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Research Fund for the Control of Infectious Diseases

Research Dissemination Reports

衛生及醫護服務研究基金
控制傳染病研究基金

研究成果報告

Cardiovascular Diseases
心血管疾病

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Immunology
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Health and Health Services Research Fund

Research Fund for the Control of Infectious Diseases

Research Dissemination Reports

EDITORIAL

We are delighted to bring you another series of dissemination reports of research projects supported by the *Research Fund for the Control of Infectious Diseases* (RFCID) and the *Health and Health Services Research Fund* (HHSRF). This issue features projects related to cardiovascular diseases, food-borne diseases and immunology. Several projects are highlighted due to their significant findings, impact on health care delivery and practice, and/or contributions to health policy formulation in Hong Kong.

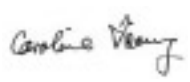
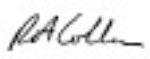
In Hong Kong, heart disease has been the second highest cause of mortality over the past few years (after cancer), and accounted for about 10% of all deaths. McGhee et al[1] developed an impact model to relate recent coronary heart disease (CHD) trends to treatments and changes in population risk factors with a view to predicting future trends. The model incorporated extensive data from a variety of sources on population demographics, morbidity and mortality figures, and health resource utilisation. The model also included data on changes in major risk factors. After extensive validation, it was inferred that up to 78% of the CHD mortality reduction between 1989 and 2001 was attributed to improvements in treatment, while 28% was related to changes in population risk factors. The fact that improvement of treatment uptake levels can have a substantial effect in reducing CHD mortality may have important implications for health planning—although considerable effort is still be needed to decrease health risks.

Developing rapid diagnostic tests that are robust enough to use with clinical samples, food and environmental samples is not a trivial undertaking. Ling[2] developed methods based on the polymerase chain reaction (PCR) and real-time (RT)-PCR for 5-hour and 3-hour detection, respectively, of salmonellae and *Vibrio cholerae* in stool, food and water samples. The RT-PCR assays for both organisms had lower limits of detection than the corresponding PCR assays. For all the assays, the limits of detection in stool samples were lower than those in food and water samples. In addition, the RT-PCR assays were cheaper than traditional culture and identification techniques. Although results were available on the same day, the overall sensitivity of the RT-PCR tests was low. Despite these promising findings, it is likely that further evaluation on a broader range of samples will be needed before routine implementation of these assays in public laboratory settings. Ultimately, such tests may be useful for the prompt identification of infections, timely control of their spread, and for epidemiological tracing of sources and contacts.

Why did some people succumb to severe acute respiratory syndrome (SARS) yet others exposed to similar or even higher risk remain unaffected? Part of the reason could be related to the innate genetic susceptibility of certain individuals. During immune responses, cytokines and chemokines are known to play important roles in antiviral activity and in cell trafficking, respectively. Lau and Peiris[3] investigated the association of polymorphisms of certain cytokine and chemokine genes with SARS. They found that one particular polymorphism of an interferon gamma allele and an allele of the chemokine RANTES were associated with SARS, and that both may affect its pathogenesis. The findings of this study may have implications for novel targets or potential mechanisms of action for future antiviral compounds.

We hope you find this selection of dissemination reports informative and enjoyable to read. These dissemination reports and the projects' full reports may be downloaded individually from the Research Fund Secretariat website (**http://www.fhb.gov.hk/grants**), where more information about the funds, including application procedures, can also be found.

Supplement co-editors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References

1. McGhee SM, So JC, Thomas GN. Explaining coronary heart disease trends in Hong Kong: creation of a model for policy and planning. Hong Kong Med J 2009;15(Suppl 2):22-5.
2. Ling JM. Rapid detection of food-borne pathogens in clinical specimens, food and environmental samples. Hong Kong Med J 2009;15(Suppl 2):26-9.
3. Lau YL, Peiris JS. Association of cytokine and chemokine gene polymorphisms with severe acute respiratory syndrome. Hong Kong Med J 2009;15(Suppl 2):43-6.

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Evaluation of energy expenditure and cardiovascular health effects from Tai Chi and walking exercise

Key Messages

1. A 12-week Tai Chi or walking exercise intervention produced significant and similar beneficial effects on body composition, aerobic fitness, muscular fitness, fasting blood glucose, resting metabolic rate, and perceived health in middle-aged Chinese.
2. While Tai Chi and walking both elicited significant cardiorespiratory responses and energy expenditure to the moderate intensity level, walking exercise elicited about 46% higher metabolic cost than Tai Chi exercise.

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Introduction

Other than cancer, cardiovascular diseases (CVD) account for major mortality and morbidity rates in Hong Kong. Increasing energy expenditure through regular exercise has been found to lower the risk of CVD and to control hyperlipidaemia and obesity. A cross-sectional survey revealed that Tai Chi (TCC) and walking exercises (WLK) are widely practised by Hong Kong citizens.[1] However, there have been limited studies to compare the health benefits of the two.

An influential medical report confirmed that daily accumulation of 30 minutes of moderate physical activity significantly lowered the risk of developing many chronic diseases. Some studies demonstrated various health benefits from regular WLK. The most recent study by Murphy et al[2] provided an excellent example. This reported that in a 6-week WLK programme (5 days per week), a single bout of continuous 30 minutes of WLK per day yielded similar health benefits to three 10-minute walk per day.

Tai Chi is an ancient form of Chinese fitness exercise. A number of studies have investigated the positive health effects of TCC for patients,[3] as well as for healthy individuals.[4] Such health benefits include improvement in: aerobic fitness and energy metabolism, muscular strength and balance, and mental control. Compared to WLK, it is intuitively perceived to be of lower exercise intensity and metabolic cost. Surprisingly, Lan et al[4] reported that the exercise intensity of a typical session of TCC (24 minutes Yang style) exceeded 70% of maximal heart rate. However, the energy cost of this single bout of TCC has not been investigated. Tai Chi and WLK seem to provide similar benefits but have not been compared simultaneously except in one study. Heart rate, blood pressure, and urinary catecholamine changes for TCC and WLK at 6 km/h are similar. However, currently there are no scientific data in this respect on Hong Kong Chinese population. Results from our study would therefore be valuable for practitioners to provide quantifiable weight control prescriptions for obese individuals, as well as for those who need to improve cardiovascular health.

Methods

This study was conducted from September 2004 to August 2006.

Subjects

A total of 374 sedentary, middle-aged subjects (men and women) from large housing estates in Shatin (New Territories, Hong Kong) who had no known cardiovascular and pulmonary diseases, neurological disorder, or musculo-skeletal disorders were recruited. Informed consent was obtained from participants prior to recruitment. Subjects were then randomly assigned into either a TCC, WLK, or control (CTL) group. To avoid contamination of recruits from excessive numbers in any one of the nine geographical locations, subjects were randomised by locations using a simple random drawing procedure. As a result, three locations were assigned TCC, three for WLK and the remaining three locations as control. For each treatment group, the minimal sample size was pre-determined at not less than 100, resulting in a total of not less than 300. Meanwhile, in order to match the age and gender distribution among the three groups, an effort was made to recruit

approximately 10 subjects of each gender and for each 5-year age-group between the ages of 36 and 60 years.

Exercise intervention

After the initial measurements of resting and exercise metabolic costs and CVD risks, the TCC and WLK groups were prescribed a 12-week training programme, with 5 days of exercise per week (led by qualified instructors for 3 days, and on their own for 2). A modified 32 Yang style TCC was selected. For the WLK group, subjects were required to walk 5 times per week. Upon completion of the 12-week exercise intervention programmes, all the tests were repeated. For the control group, the pre- and post-exercise assessments were conducted in the same way, however, there was no exercise intervention.

Measurement of energy expenditure

All subjects were instructed to lie on a bed for 20 minutes in an environment with a comfortable temperature and humidity. Resting metabolic rate, in terms of oxygen consumption (VO_2 in mL/kg body weight/min), and energy expenditure (KCal in KCal/min), were measured by the Cosmed K4b2 metabolic measuring system. The lowest metabolic value for a continuous 10-minute period was taken to be the resting metabolic rate. To compare the metabolic cost between TCC and WLK, another 30 TCC practitioners of similar age as the intervention participants were recruited to perform 10 min of TCC, 10 min of WLK in self-selected pace, and 10 min of WLK at a controlled heart rate similar to those encountered with TCC. Each form of exercise was performed three times in a random order. Metabolic cost, in terms of VO_2, KCal, and heart rate (HR) were measured using the Cosmed K4b2 analyser.

Measurement of cardiovascular disease risks

These risk factors were determined by blood tests (total, low- and high-density lipoprotein cholesterols, triglycerides, fasting blood glucose). Body composition was measured by bioelectrical impedance analysis. Criteria of CVD risks were adopted from the American Heart Association and the American College of Sports Medicine. Cardio-respiratory fitness, in terms of VO_2max, was measured using a symptom limited treadmill exercise test. Subjects were also required to answer a 'typical 1-week food frequency' questionnaire for diet analysis.

Other measures

Perceived health status was measured by a Chinese version Short-form (12 items) Health-related Quality of Life questionnaire. Six months after the intervention, exercise compliance after the cessation of the 12-week exercise training programme was enquired into by a questionnaire.

Statistical analysis

Age-adjusted repeated measures multivariate analysis of covariance, and subsequent univariate analysis of covariance and Scheffee tests were performed to examine changes in outcome measures between TCC, WLK and CTL groups.

Results

Descriptive statistics

Upon recruitment, there were 129 TCC, 121 WLK and 124 CTL participants. Due to voluntary drop-out and elimination of subjects with low attendance (<70%) in classes, the final sample size for analyses entailed 104 TCC (completion rate 81%), 91 WLK (completion rate 75%), and 121 CTL (completion rate 98%) participants.

Body composition

Statistically significant reductions in body composition measures (body weight, body mass index [BMI], waist circumference, hip circumference, waist:hip ratio, % body fat, and sum of skinfolds) for both WLK men ($P<0.05$ to $P<0.001$) and women ($P<0.01$ to $P<0.001$) were noted. Similar findings were observed for waist circumference, waist:hip ratio, % body fat, and sum of skinfolds in both TCC men ($P<0.001$) and women ($P<0.001$). In addition, TCC men had significant body weight and BMI reductions ($P<0.001$), while the reductions for TCC women were not significant. By contrast, most of the body composition measures in the CTL group increased slightly, although not to a statistically significant extent. Waist ($P<0.01$) and hip ($P<0.001$) circumference, and % body fat ($P<0.05$) of CTL men increased significantly. Sum of skinfolds in CTL women decreased slightly ($P<0.05$). The pre-post changes in BMI for the subjects are shown in Figure 1.

Physical fitness

For men, items that showed improvements post exercise were: back lift strength ($P<0.01$ for TCC and $P<0.01$ for WLK); right leg balance ($P<0.05$ for WLK only); curl-up ($P<0.001$ for both TCC and WLK); and sum of sit-and-reach ($P<0.001$ for both TCC and WLK). For women, corresponding items showing improvements were: back lift strength ($P<0.001$ for WLK only); sum of balance test ($P<0.01$ for WLK only); curl-up ($P<0.01$ for TCC and $P<0.05$ for WLK); and sum of sit-and-reach ($P<0.001$ for TCC, $P<0.01$ for WLK). For CTL men, diastolic blood

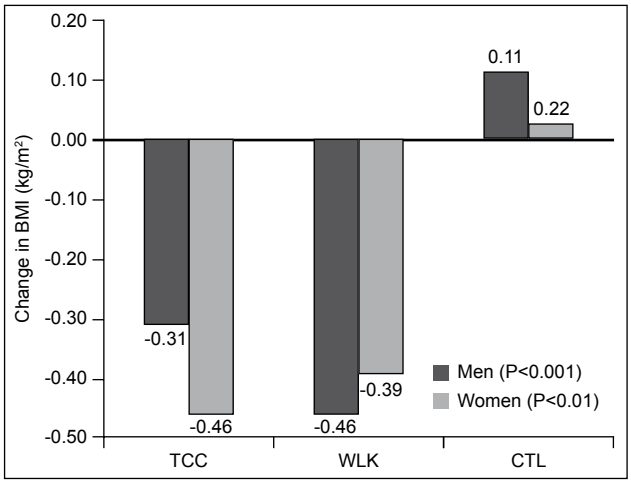

Fig 1. Mean changes in body mass index (BMI) in Tai Chi (TCC), walking exercises (WLK) and control (CTL) subjects

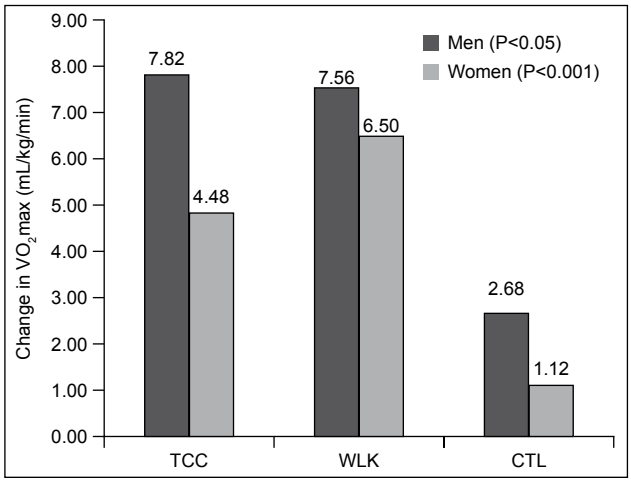

Fig 2. Mean changes in VO_2max in Tai Chi (TCC), walking exercises (WLK) and control (CTL) subjects

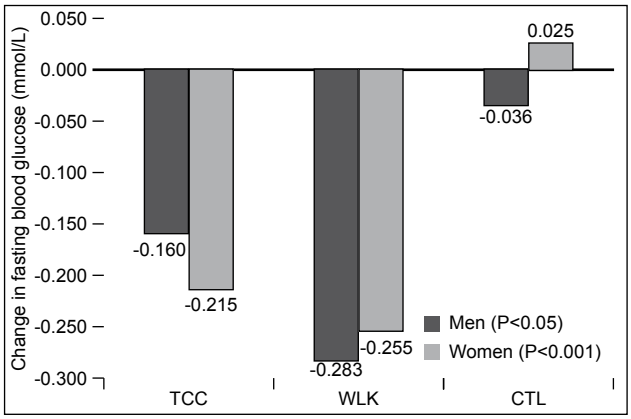

Fig 3. Mean changes in fasting blood glucose in Tai Chi (TCC), walking exercises (WLK) and control (CTL) subjects

pressure (DBP) (P<0.01) and leg lift (P<0.01) decreased slightly, whereas arm lift, back lift and curl-up increased slightly (P<0.01). For CTL women, DBP (P<0.05), arm lift (P<0.01) and shoulder lift (P<0.05) decreased slightly, but back lift (P<0.001) and curl-up (P<0.05) increased slightly. Post-hoc pairwise comparison suggests that both TCC and WLK improved hamstring flexibility compared to CTL as reflected by changes in sit-and-reach scores. However, post-hoc comparison showed non-significant difference between TCC and WLK subjects.

Aerobic fitness

The VO_2max for both exercise groups improved significantly after the exercise (P<0.001 for all TCC and WLK subjects) when compared to the CTL group (Fig 2). Post-hoc comparison showed non-significant difference between TCC and WLK participants.

Resting energy expenditure

There were significant increases in resting energy expenditure (REE)–VO_2 (mL/min/kg) [P<0.001] and REE-KCal (KCal/min) [P<0.01] post exercise, in both TCC and WLK men in comparison to CTL men. No such trend was observed for women.

Blood profiles

In both TCC and WLK men and women, fasting blood glucose levels decreased significantly post exercise (P<0.001, Fig 3).

Dietary intakes

In both men and women, the KCal intake from all of carbohydrates, fat and protein showed no significant differences post exercise, except that for TCC women KCal intake from protein was higher (P<0.01).

Changes in perceived health

The SF-12 questionnaire showed that there was generally an improvement of perceived health status in both TCC and WLK subjects. No such trend was observed for the CTL subjects.

Six-month maintenance

In the respective TCC and WLK subjects, 64% and 77% of the subjects continued to perform their TCC and WLK exercises in the ensuing 6 months, 60% and 68% did so in the ensuing month, and 53% and 65% did so in the ensuing week. Regarding the respective mean frequencies of exercise being performed per week, they were: 2.5 and 3 times in the ensuing 6 months, 2.1 and 2.8 times in the ensuing month, and 2.2 and 2.8 times in the ensuing week.

Metabolic cost

To evaluate the metabolic cost of TCC and WLK, 30 more TCC practitioners were recruited to perform three types of exercises in a randomised order: (1) simplified 33 Yang style TCC performed at a regular pace; (2) brisk walking (BW) at a self-selected pace (WLK-BW); and (3) walking under controlled heart rate (HRC) similar to the TCC exercise (WLK-HRC). Repeated measures analysis of variance revealed that VO_2, HR, ratio of work metabolic rate to resting metabolic rate (MET) and energy expenditure (EE) in the WLK-BW group were significantly greater than those in the TCC and WLK-HRC (P<0.05, Table) groups, whereas no differences were noted between the TCC and WLK-HRC (P>0.05) groups. The exercise HRs for TCC, WLK-BW, and WLK-HRC were about 56%, 65%, and 57% of maximum, respectively. These figures revealed that WLK-BW produced approximately a 46% higher metabolic cost than TCC. Post hoc comparison showed non-significant difference between TCC and WLK-HRC groups. No interaction was found for women. Notably, TCC, WLK-BW, and WLK-HRC elicited significant cardiorespiratory and EE responses to the moderate intensity exercise.

Discussion

This study is perhaps the first to provide a comprehensive comparison of health and fitness in middle-aged, Chinese TCC and WLK subjects. Encouragingly, both 3-month TCC and WLK exercise training produced similar levels of weight reduction, improvement in blood profile and physical fitness, and significantly increased the resting metabolic rate. Both TCC and WLK resulted in reductions

Table. Cardiorespiratory and energy expenditure responses in Tai Chi (TCC), brisk walking (WLK-BW), and walking under controlled heart rate (WLK-HRC) subjects

Measurement*	TCC	WLK-BW	WLK-HRC
VE (mL/min)	18.6 ± 4.1	29.3 ± 7.4†	21.7 ± 5.4
VO_2 (mL/min)	681.9 ± 183	993 ± 279.8†	731.6 ± 238.6
VO_2 (mL/kg/min)	11.3 ± 2.5	16.6 ± 4.2†	12.2 ± 3.7
EE_{total} (KCal)	32.8 ± 8.9	48.1 ± 13.4†	34.8 ± 11.3
EE (KCal/min)	3.2 ± 0.9	4.8 ± 1.3†	3.5 ± 1.1
METS	3.24 ± 0.7	4.7 ± 1.2†	3.5 ± 1.0
$HR_{exercise}$ (bpm)	98 ± 16	114 ± 16†	100 ± 15
RER	0.82 ± 0.09	0.84 ± 0.07†	0.8 ± 0.09
RPE	10.1 ± 1.1	11.2 ± 1.4	10.3 ± 1.0

* VE (mL/min) denotes minute ventilation, VO_2 (mL/min) minute oxygen uptake, VO_2 (mL/kg/min) minute oxygen uptake relative to each kg of body weight, EE_{total} (KCal) total energy expenditure for 10 min of exercise, EE (KCal/min) total energy expenditure per minute, METS the ratio of work metabolic rate to the resting metabolic rate, $HR_{exercise}$ (bmp) heart rate in exercise (beats per minute), RER respiratory exchange ratio, RPE rate of perceived exertion on a 6-20 scale

† P<0.05, TCC versus WLK-BW, WC versus WLK-HRC

of approximately 1 kg in body weight and 2.5 cm in waist circumference in men. In women, corresponding reductions were 0.33 kg after TCC and 0.87 kg after WLK and about 5 cm of waist circumference for both forms of exercise. Similar significant reductions in % body fat and sum of skinfolds were also noted after TCC and WLK in both men and women. In all three groups, some muscular strength tests and curl-up endurance improved in both men and women, however there was no significant interaction. These improvements were probably due to enhanced experiences compared to the pre-exercise status. However, some muscular strength tests in CTL subjects revealed significant decreases (leg lift in men, and arm and shoulder lifts in women). Only the interaction of sit-and-reach flexibility was significant, which suggested that both TCC and WLK improved hamstring flexibility compared to CTL activity. Regarding aerobic fitness in men, after TCC VO_2max improved 22% and after WLK it improved 21%. In women, corresponding figures were 15% after TCC and 20% after WLK. Similar results were noted for changes in fasting blood glucose. More importantly, other than the physiological parameters described above, perceived health status also improved significantly (24-29% after WLK, and 13-14% after TCC). Moreover, 60% to 70% of the exercise participants continued to practise regular exercise training 6 months after the intervention.

Regardless of the similar levels of health improvement from TCC and WLK, the mean exercise HR was 33% higher in WLK than TCC in men, and 34% higher in women. The experiment in metabolic cost comparison revealed that WLK elicited 46% higher VO_2 and EE. When exercise intensity and safety is a concern, TCC appears more desirable than WLK, since it elicits lower metabolic demands but yields similar levels of health benefits. Why TCC produces similar health and fitness benefits at a lower metabolic demand compared to WLK is not known. However, it is the belief of Chinese martial arts practitioners that there is an internal energy called 'qi' that circulates inside the body when practising Tai Chi. The slow and regular breathing technique combined with slow but steady muscular movement is believed to produce 'qi' that stimulates long-term changes in physical fitness.

Although TCC elicits lower metabolic demand, the present study recorded that it produced 56% of age-predicted HR max and 3.3 METs (VO_2=11.5 mL/kg/min) of exercise intensity, which is considered to be an aerobic exercise at a moderate intensity. Li et al[5] reviewed 31 TCC studies and found that nine of them were reported to entail moderate intense exercise, with no more than 55% of VO_2max. The present study recorded that the exercise HR following TCC ranged from 86 to 98 bpm, which are fairly consistent with the findings in a previous study recording a peak HR of 95-98 bpm after TCC.[6]

Although the present study reports a number of significant health improvements from TCC and WLK, arguably the magnitude of improvement was small. It is important to note that, in both the TCC and WLK interventions, the exercise volume was not large; the active exercise time was only 30 minutes per session, 3 times a week and its intensity was only low to moderate. Tai Chi is a low-intensity activity, which yields significant health improvement similar to WLK. This result provides insight showing that even a low intensity of activity (~3 METs, 56% HR max) produces significant health improvement.

The present study reports similar health and fitness improvement for TCC and WLK. However, when the magnitudes of the measured variables are observed, some parameters after WLK did elicit slightly higher improvements than TCC, although not statistically significant. The present study failed to reveal improvements in blood lipid profiles. The latter findings should be viewed as pertaining to relatively short periods of exercise intervention; limited studies have found changes in blood lipid profiles associated with TCC. Further studies with longer intervention periods are suggested.

Acknowledgement

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References

1. Hong Kong Sports Development Board. Sports Participation Survey 2000. Hong Kong: Hong Kong Sports Development Board; 2002.
2. Murphy M, Nevill A, Neville C, Biddle S, Hardman A. Accumulating brisk walking for fitness, cardiovascular risk, and psychological health. Med Sci Sports Exerc 2002;34:1468-74.
3. Channer KS, Barrow D, Barrow R, Osborne M, Ives G. Changes in haemodynamic parameters following Tai Chi Chuan and aerobic exercise in patients recovering from acute myocardial infarction. Postgrad Med J 1996;72:349-51.
4. Lan C, Lai JS, Chen SY, Wong MK. 12-month Tai Chi training in the elderly: its effect on health fitness. Med Sci Sports Exerc 1998;30:345-51.
5. Li JX, Hong Y, Chan KM. Tai chi: physiological characteristics and beneficial effects on health. Br J Sports Med 2001;35:148-56.
6. Gong LS, Qian JA, Zhang JS, et al. Changes in heart rate and electrocardiogram during Taijiquan exercise: analysis by telemetry in 100 subjects. Chin Med J (Engl) 1981;94:589-92.

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Translation and validation of two Chinese health-related quality of life instruments in patients with coronary heart disease

Key Messages

1. The Chinese version of the 27-item MacNew health-related quality of life (HRQL) questionnaire is a valid, reliable and responsive core coronary heart disease (CHD)–specific HRQL measure. It can be used to compare the health outcomes, burdens of illness, and treatment effectiveness in pure or mixed populations of patients with myocardial infarction, angina, or heart failure in clinical trials and in routine clinical practice.
2. The Chinese version of the 35-item Myocardial Infarction Dimensional Assessment Scale (MIDAS) did not perform as well. Although four of the seven subscales, which cover the physical and psychosocial aspects of HRQL, are psychometrically sound when used to evaluate HRQL among CHD patients with different cardiac diagnostic categories, the remaining three subscales covering treatment-related aspects are not. The latter had only weak validity and responsiveness, which may be due to cultural differences.
3. To improve the overall performance of the Chinese version of the MIDAS, further effort is required to clarify the treatment-related impact of CHD on well being from the patient's perspective.

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Introduction

The prevalence of coronary heart disease (CHD) is increasing in Asia, including Hong Kong and mainland China, where it is a major cause of death and disability.[1] Patients with CHD are typically diagnosed by physician with one or more of three inter-related (but clinically distinct) conditions: myocardial infarction (MI), angina pectoris, or heart failure. This diagnostic conceptualisation has led to important increases in specific treatments with researchers increasingly focusing their attention on comparing the efficacy of one intervention with another among homogeneous groups of patients meeting explicit diagnostic criteria. Comparing the burden of illness and treatment effectiveness for CHD across the spectrum of patients with frequently co-occurring conditions, such as MI, angina pectoris or heart failure, requires a common outcome measure. Yet, a major limitation of existing condition-specific health-related quality of life (HRQL) instruments is that they are not suitable for making comparisons across different CHD diagnoses.

The purpose of this study was to translate the MacNew health-related quality of life questionnaire[2] (MacNew) and the Myocardial Infarction Dimensional Assessment Scale[3] (MIDAS) into Chinese, and to examine their psychometric properties in Chinese patients with differential diagnoses of CHD, including MI, angina pectoris or heart failure.

Methods

Study design

This was a longitudinal study.

Subjects and settings

A convenience sample of 398 patients with evidence of CHD was recruited from the cardiac unit of a regional hospital between December 2004 and February 2006. Of these, 365 (MI: 117; angina: 154; heart failure: 94) completed all the study instruments; 92 of the latter were randomly selected for 7-day post-test assessment with the tested instruments, and the 3-month repeat data collection was completed in 363. The mean age of the patients was 65 (standard deviation, 12) years, with more heart failure patients being older (P=0.009). The male-to-female ratio was lower among patients with heart failure (2:1) than MI or angina (4:1).

Main study instruments

The 27-item C-MacNew and the 35-item C-MIDAS were translated from their original English versions by using Brislin's model of forward and backward translation. Based on a 7-point and 5-point Likert scale, the C-MacNew and the C-MacNew examine CHD disease–specific quality of life in three (physical, emotional, and social) and seven (physical activity, insecurity, emotional reaction, dependency, diet, concerns over medication, side-effects) dimensions, respectively. Previous studies have demonstrated high validity and reliability for both of these instruments.

Table 1. Reliability of Chinese versions of MacNew health-related quality of life questionnaire (C-MacNew) and the Myocardial Infarction Dimensional Assessment Scale (C-MIDAS)

Instruments	Myocardial infarction	Angina	Heart failure
C-MacNew			
Overall scale	0.91	0.94	0.94
Physical	0.86	0.89	0.88
Emotion	0.90	0.92	0.92
Social	0.88	0.91	0.90
C-MIDAS			
Overall scale	0.94	0.95	0.93
Physical activity	0.90	0.92	0.87
Insecurity	0.95	0.94	0.92
Emotional reaction	0.86	0.90	0.88
Dependency	0.78	0.79	0.65
Diet	0.88	0.85	0.90
Concerns over medication	0.79	0.82	0.70
Side-effects	0.70	0.77	0.67

Translation and validation plan

1. The reliability of the C-MacNew and the C-MIDAS was determined by examining their internal consistency and 7-day test-retest reliability with Cronbach's alpha and intraclass correlation coefficient, respectively.
2. Construct validity of the C-MacNew and the C-MIDAS was established by computing their correlations with the Short-Form 36-item Health Survey (SF-36) and the Hospital Anxiety and Depression Scale (HADS). Confirmatory factor analysis was also used to determine whether the C-MacNew and the C-MIDAS conform to the factor structure of their respective original versions.
3. Discriminatory validity was determined by using the logic of 'known-groups' approach, using age, gender, the presence or absence of anxiety and depression according to the HADS score, and perceived health deterioration (according to the health transition item of the SF-36) as discriminative variables.
4. Longitudinal validity was determined by examining the correlations of the changes in the C-MacNew and the C-MIDAS scores with the changes in the SF-36 scores over a 3-month period.
5. Responsiveness of the C-MacNew and the C-MIDAS was determined by computing the effect size and standardised response mean for the changes of scores over a 3-month period.

RESULTS

Reliability

The results suggest good internal consistency for the C-MacNew and the C-MIDAS in measuring HRQL for patients with different cardiac diagnostic categories, though the Cronbach's alphas for the 'side-effects' and 'dependency' subscales of the C-MIDAS were slightly lower than the criterion level in heart failure patients (Table 1). Both the instruments are reproducible, with intraclass correlation coefficient ranged from 0.88-0.93 and 0.72-0.92, respectively.

Validity

The construct validity of the C-MacNew and the C-MIDAS (except the 'diet', 'concerns over medication' and 'side-effects') was supported by their significant moderate-to-strong correlations with both SF-36 physical and mental component scores and the HADS anxiety and depression scores (Table 2). Results of confirmatory factor analysis also indicated that the C-MacNew (χ^2/df=1.41, RMSEA=0.043, NFI=0.93, NNFI=0.94 and CFI=0.95) and C-MIDAS (χ^2/df=2.32, RMSEA=0.059, NFI=0.94, NNFI=0.95, CFI=0.96) conformed to the original 3-factor and 7-factor structure, respectively. However, the measurement model of the C-MacNew suggested that there was only one item (instead of 12 items in the original version) that loaded significantly onto more than one subscale, whereas that of the C-MIDAS suggested the existence of error covariance between item 2 (had angina symptom) and item 3 (had angina that affected life).

The results of discriminative validity indicated that both the C-MacNew and the C-MIDAS (except for 'diet', 'concerns over medication', 'side-effects' subscales) identified poorer HRQL in MI or angina patients who reported anxiety and perceived deteriorated health. Both instruments also identified a significantly poorer HRQL in female patients with angina. As for heart failure patients, the C-MacNew and two subscales of the C-MIDAS scores (ie 'physical activity' and 'insecurity') indicated significantly poorer HRQL in patients who were female, at old age, with anxiety and with perceived health deterioration.

Longitudinal validity of the C-MacNew and the C-MIDAS was also established as the changes in the majority of the subscales' scores showed a significant and moderate relationship with the changes in the SF-36 physical and mental component scores over a 3-month period. Nevertheless, three C-MIDAS subscales which had low discriminative validity (ie 'diet', 'concerns over medication', 'side-effects') also had poor performance on longitudinal validity testing (Table 3).

Responsiveness

The mean changes in the C-MacNew and C-MIDAS scores were statistically significant (P<0.001) in the three cardiac diagnostic groups. The results indicated a moderate-to-strong responsiveness of C-MacNew in detecting changes in HRQL (effect size: 0.51-0.78; standardised response mean: 0.53-0.78) in all the three cardiac diagnostic groups. This was also true for most of the C-MIDAS subscales (effect size: 0.43-0.94; standardised response mean: 0.46-0.96), with the exception of 'concerns over medication' and 'side-effects' subscales (effect size, 0.20-0.38; standardised response mean, 0.21-0.37).

Discussion

This study substantiates previously published psychometric data on the original versions of MacNew and MIDAS

Table 2. Construct validity of Chinese versions of MacNew health-related quality of life questionnaire (C-MacNew) and the Myocardial Infarction Dimensional Assessment Scale (C-MIDAS)

Instruments	Myocardial infarction	Angina	Heart failure
Correlation with Short-Form 36-item Health Survey (SF-36) physical component scores			
C-MacNew: physical	0.60‡	0.62‡	0.64‡
C-MacNew: emotional	0.48‡	0.47‡	0.50‡
C-MacNew: social	0.55‡	0.55‡	0.57‡
C-MIDAS: physical activity	-0.61†	-0.69†	-0.71†
C-MIDAS: insecurity	-0.43†	-0.56†	-0.54†
C-MIDAS: emotional reaction	-0.28†	-0.39†	-0.41†
C-MIDAS: dependency	-0.43†	-0.47†	-0.50†
C-MIDAS: diet	-0.17§	-0.12§	-0.04§
C-MIDAS: concerns over medication	-0.22*	-0.16§	-0.07§
C-MIDAS: side-effects	-0.29†	-0.29†	-0.07§
Correlation with SF-36 mental component scores			
C-MacNew: physical	0.58‡	0.56‡	0.66‡
C-MacNew: emotional	0.59‡	0.59‡	0.70‡
C-MacNew: social	0.56‡	0.57‡	0.66‡
C-MIDAS: physical activity	-0.54†	-0.63†	-0.58†
C-MIDAS: insecurity	-0.44†	-0.62†	-0.58†
C-MIDAS: emotional reaction	-0.46†	-0.53†	-0.58†
C-MIDAS: dependency	-0.48†	-0.46†	-0.46†
C-MIDAS: diet	-0.22*	-0.07§	-0.12§
C-MIDAS: concerns over medication	-0.30†	-0.13§	-0.27†
C-MIDAS: side-effects	-0.29†	-0.16*	-0.26†
Correlation with Hospital Anxiety and Depression Scale (HADS) anxiety score			
C-MacNew: physical	-0.44‡	-0.47‡	-0.37‡
C-MacNew: emotional	-0.67‡	-0.68‡	-0.59‡
C-MacNew: social	-0.47‡	-0.50‡	-0.44‡
C-MIDAS: physical activity	0.39‡	0.38‡	0.32†
C-MIDAS: insecurity	0.59‡	0.56‡	0.51‡
C-MIDAS: emotional reaction	0.58‡	0.49‡	0.46‡
C-MIDAS: dependency	0.49‡	0.46‡	0.45‡
C-MIDAS: diet	-0.06§	-0.01§	-0.20§
C-MIDAS: concerns over medication	0.28†	0.21†	0.16§
C-MIDAS: side-effects	0.17§	0.25†	0.23*
Correlation with HADS depression score			
C-MacNew: physical	-0.53‡	-0.55‡	-0.61‡
C-MacNew: emotional	-0.80‡	-0.75‡	0.70‡
C-MacNew: social	-0.56‡	-0.59‡	-0.67‡
C-MIDAS: physical activity	0.47‡	0.47‡	0.57‡
C-MIDAS: insecurity	0.62‡	0.54‡	0.53‡
C-MIDAS: emotional reaction	0.53‡	0.34‡	0.36‡
C-MIDAS: dependency	0.48‡	0.37‡	0.49‡
C-MIDAS: diet	0.02§	-0.02§	0.02§
C-MIDAS: concerns over medication	0.36‡	0.18*	0.05§
C-MIDAS: side-effects	0.23*	0.25†	-0.01§

* P<0.05

† P<0.01

‡ P<0.001

§ Not significant

Table 3. Longitudinal validity of Chinese versions of MacNew health-related quality of life questionnaire (C-MacNew) and the Myocardial Infarction Dimensional Assessment Scale (C-MIDAS)

Instruments	Changes in Short-Form 36-item Health Survey					
	Physical component score			Mental component score		
	Myocardial infarction	Angina	Heart failure	Myocardial infarction	Angina	Heart failure
Changes in C-MacNew						
Physical	0.63‡	0.66‡	0.65‡	0.53‡	0.59‡	0.58‡
Emotion	0.58‡	0.48‡	0.51‡	0.58‡	0.58‡	0.61†
Social	0.58‡	0.62‡	0.54‡	0.55‡	0.56‡	0.62‡
Changes in C-MIDAS						
Physical activity	-0.61‡	-0.57‡	-0.57‡	-0.47‡	0.60‡	0.50‡
Insecurity	-0.31‡	-0.45‡	-0.45‡	-0.30†	0.58‡	0.47‡
Emotional reaction	-0.26†	-0.33‡	-0.40‡	-0.34‡	0.48‡	0.41‡
Dependency	-0.24*	-0.41‡	-0.52‡	-0.39‡	0.30‡	0.35†
Diet	-0.93§	0.05§	-0.01§	-0.12§	0.13§	0.25*
Concerns over medication	-0.22*	-0.02§	-0.24*	-0.22*	0.10§	0.31†
Side-effects	-0.16§	-0.06§	-0.12§	-0.26†	0.12§	0.20§

* P<0.05

† P<0.01

‡ P<0.001

§ Not significant

in CHD patients. Both of these instruments are internally consistent and reproducible in Chinese patients in each of the three CHD diagnostic groups (MI, angina, and heart failure). Their factor structures are similar to those of their respective original versions for measuring the various aspects of HRQL among CHD patients. C-MacNew and four of the seven subscales of C-MIDAS (physical activity, insecurity, emotional reactions, dependency) demonstrated good construct and longitudinal validity. They are also responsive to detecting changes in HRQL in the three cardiac diagnostic groups. All these findings provide strong evidence to suggest that the C-MacNew may have value as a core questionnaire in patients with a differential diagnosis of CHD.

As for C-MIDAS, the poor performance of the three subscales (diet, concerns over medication and side-effects) may be related to concerns about risk factors for CHD and worries about medical treatment that patients regard as less important in interpreting disease impact and life situations.[4] Some studies also found that CHD patients did not consider adjusting their lifestyle in order to reduce CHD risk factors as important. Such an attitude might be more prominent in the current sample, as older people in Chinese culture tend to adopt a 'do nothing approach' and allow fate to take its course.[5] As for the 'side-effects' subscale, the items such as 'felt the cold more' and 'unwanted side-effect' might not be specific enough to assess patients' problems associated with medical treatment. Although the study provides evidence of adequate psychometric properties of only four of the Chinese MIDAS subscales, these four subscales provide a wide coverage of the physical and psychosocial health and functioning of CHD patients. As the original MIDAS suggests the use of subscale scores, these subscales can be used as an independent, reliable, valid and responsive core CHD-specific HRQL measure among patients in different diagnostic categories. Improving the performance of the Chinese MIDAS requires further effort to clarify patients' perspectives of treatment-related impact of CHD on well being.

This study has limitations. First, recruiting a consecutive sample of CHD patients managed in a single acute care setting may limit the generalisability of the findings for patients in community-dwelling or rehabilitative settings. Second, the over-representation of male patients in the sample further threatens the external validity of the findings. Finally, as we only collected 7-day post-test data from 25% of the patients, the reproducibility of the C-MacNew and the C-MIDAS could not be examined for each cardiac diagnostic group individually.

Conclusions

In conclusion, there is sufficient evidence that the psychometric properties of the C-MacNew and some of the subscales of the C-MIDAS are adequate to warrant recommending these HRQL instruments for Chinese patients with MI, angina or heart failure as an outcome measure to enhance treatment evaluation for patients with CHD. They can be used to compare health outcomes, burden of illness, and treatment effectiveness on pure or mixed populations with the three previously mentioned cardiac diagnoses.

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References

1. Death rates for leading causes, 2001-2007. Centre for Health Protection, Department of Health, The Government of the Hong Kong Special Administrative Region. http://www.chp.gov.hk/data.asp?lang=en&cat=4&dns_sumID=117&id=27&pid=10&ppid. Accessed 14 October 2008.
2. Lim LL, Valenti LA, Knapp JC, et al. A self-administered quality-of-life questionnaire after acute myocardial infarction. J Clin Epidemiol 1993;46:1249-56.
3. Thompson DR, Jenkinson C, Roebuck A, Lewin RJ, Boyle RM, Chandola T. Development and validation of a short measure of health status for individuals with acute myocardial infarction: the myocardial infarction dimensional assessment scale (MIDAS). Qual Life Res 2002;11:535-43.
4. Emslie C. Women, men and coronary heart disease: a review of the qualitative literature. J Adv Nurs 2005;51:382-95.
5. Webster R. The experiences and health care needs of Asian coronary patients and their partners. Methodological issues and preliminary findings. Nurs Crit Care 1997;2:215–23.

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A pilot study to examine the feasibility and acceptability of a community model for exercise prescription for patients with chronic disease

Key Messages

1. A model of community care for chronic obstructive pulmonary disease (COPD) and chronic heart failure (CHF) that incorporates exercise prescription is lacking, although the benefits of exercise for these diseases are established.
2. Group programmes incorporating exercise, disease education, and social support consisting of weekly sessions for 12 weeks were designed for COPD and CHF patients, in groups of 8 to 10. A home exercise programme was also prescribed.
3. This model was feasible, enjoyed good compliance, improved symptoms and measures of psychosocial outcome for both disease and improved exercise tolerance in the CHF group.
4. This model could be further developed as an integral part of community management for patients with chronic diseases.

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Introduction

Chronic diseases such as osteoarthritis, heart disease, chronic obstructive airways disease, and diabetes mellitus, account for a large proportion of the Hong Kong Hospital Authority's health care expenditure. For example, during 1997, chronic obstructive airways disease accounted for the largest number of bed days occupied (BDO) in its acute hospitals, and the second largest number of BDO in all types of its hospitals. Ischaemic heart disease, heart failure, and diabetes ranked 5th, 6th, and 7th highest in terms of BDO for acute hospitals. Although accounting for half the BDO compared with diabetes mellitus, osteoarthritis is a common condition affecting the elderly population that gives rise to disability. With the ageing of the population, problems with mobility are also prevalent. Currently there is an emphasis on pharmacological treatment for chronic diseases for which there is no cure, when the approach should be to maximise the remaining quality of life. This might be achieved through promoting the capacity for independent living and social functioning, as well as psychosocial well being. Although the benefits of exercise in chronic disease prevention are well known, the benefits of physical activity among those with established diseases are not widely appreciated. Thus, in general an exercise prescription is seldom incorporated as part of chronic disease management. Currently, exercise forms part of short-term, hospital rehabilitation-based programmes for stroke, myocardial infarction, chronic obstructive lung disease, and osteoarthritis. In reality, exercise prescription should be applied to a wider spectrum of patients on a continuing basis as part of their therapy. Patients with the above chronic heart failure, diabetes mellitus, as well as the frail elderly with mobility problems merit exercise prescriptions.[1] Thus, improvements in exercise tolerance as well as psychological and social well being have been achieved in patients with chronic obstructive airways disease as well as heart failure.[1] This is in addition to the use of exercise in health promotion for disease prevention. However, it is uncertain how this should be incorporated into the disease management programme. Questions such as the site (home versus health care facility), the contents of the programme, whether it should be a group or individual programme, have largely been unexplored. A key target for any model of exercise prescription should be to motivate patients to persevere with such programmes. Therefore, any model should include characteristics that are likely to encourage compliance. The assumption of primary care patient services by the Hospital Authority in 2003 provides an opportunity to develop and test such a model for incorporating exercise prescriptions into the management of chronic diseases and frailty. Although this model of service provision has theoretical benefits, it is not known how the public or health care professionals may perceive its usefulness. Moreover, there is little information regarding the feasibility and possible benefits of such a model. Before this model is incorporated into existing services, a pilot study is needed to test its feasibility, with a view to a subsequent larger study to evaluate its effectiveness.

Aims

- To develop a model for community management of chronic disease and frailty, that incorporates exercise prescription, using chronic obstructive pulmonary disease (COPD) and chronic heart failure (CHF) as examples;

- To test its feasibility in community centres linked to COPDs;
- To determine patient response using quantitative and qualitative methods; and
- To document means of measuring physical performance as well as psychosocial well being before and after the programme.

Subjects and methods

This study was conducted from September 2004 to February 2005. Ambulant patients with COPD and CHF living in the community and able to go outdoors were targeted. From among these, patients admitted to hospital at least once in the past 12 months were recruited from (i) the medical wards of the Prince of Wales Hospital, Shatin Hospital, (ii) out-patient clinics, and (iii) enhanced home care facilities.

Subjects who were dyspnoeic at rest or on the slightest exertion (eg getting out of bed), who could not walk or follow instructions (eg due to dementia); who had uncontrolled angina; resting systolic blood pressure of >180 mm Hg or resting diastolic blood pressure of >100 mm Hg, resting tachycardia >100 bpm, unstable or acute heart failure were excluded. Patients with acute systemic illness (eg pneumonia), uncontrolled visual or vestibular disturbances, and any recent injurious fall were also excluded.

Exercise programmes

Although patients may be taught exercise routines to be carried out at home, we hypothesised that compliance may be better if conducted with a group in a community centre, since group settings could promote social interaction, simultaneously act as a chronic disease mutual support system, and allow regular contact with health carers. The programme was designed by a team of doctors, nurses and physiotherapists. In general aerobic exercise routines were suitable for improving cardiorespiratory function,[2] while resistance exercises were suitable for improving muscle strength and balance,[3] and exercise in general had metabolic benefits. In order to improve compliance, the exercise programme was devised to be enjoyable. For example, in a previous study it has been shown that walking exercise had a higher dropout rate than Tai Chi. Each disease group consisted of five to 10 patients, and was led by a trained research assistant, and two sessions per week (for each disease) were held, for a total of 12 weeks. At least three groups were held for each disease. Patients were also encouraged to carry out exercises on their own for the rest of the week.

Chronic obstructive pulmonary disease programme

Baseline measurements

These consisted of St Georges Respiratory Questionnaire,[4] the General Health Questionnaire (to assess subject knowledge regarding the disease), lung function measurements (FEV1, FVC), and the 6-minute walk test.

Intervention

This consisted of an educational talk, a group discussion, group exercises (warm up, breathing, free arm raising and sit-stand exercises with and without resistance, aerobic activities such as dancing), and weekly review of exercises carried out at home.

End of intervention assessment

Primary outcome measures

These were related to compliance (attendance rate at group sessions, number of days of exercise at home [recorded in a diary]) and patients' view of the service.

The patients' view of the usefulness of the service in relation to their functional ability, symptoms, general well being, intention to continue the programme, and value of mutual support were sought, using a structured questionnaire as well as by running focus groups. The focus group evolved as part of the last session. The following areas were explored with the group: the reason for agreeing to join the programme, comments on the running of the programmes (positive and negative aspects), and their perspective of the benefits of the programme itself. In addition, other spontaneous comments were also entertained from the participants. The discussions were recorded and then transcribed. Common themes were identified, and the responses grouped according to the categories. Additional comments that did not fall into these common themes were also listed.

Secondary outcome measures

These included repeat of baseline measurements to assess any symptom improvement, imporvement in general health, disease knowledge and exercise tolerance.

Chronic heart failure programme

Baseline measurements

These consisted of the CHF questionnaire, the test of knowledge regarding heart failure, the Social Support Survey Questionnaire,[5] and the Hospital Anxiety Depression Scale. These scales have already been used in an ongoing community study to evaluate the effect of relaxation therapy in CHF patients admitted to hospital. Comparison of results from the proposed study with the ongoing study can give some indication of the representativeness of the subjects, an important consideration pertinent to the small sample size of this pilot study. Body weight and blood pressure were recorded; body weight being an indicator of fluid balance, an important parameter related to the control of heart failure. The 6-minute walk test was used as an indicator of exercise tolerance, and biceps and quadriceps strength were measured using a dynamometer.

Intervention

This was similar to the programme for COPD, but with

Table 1. Comparison of psychological status at baseline and at the 12-week follow-up

Questionnaire*	Baseline† (n=33)	12-week follow-up† (n=33)	P value
GHQ- somatic symptoms domain	4.15 (2.54)	2.36 (1.82)	<0.001
GHQ- anxiety and insomnia domain	4.00 (2.96)	1.82 (1.93)	<0.001
GHQ- social dysfunction domain	8.85 (3.36)	6.48 (1.94)	<0.001
GHQ- depression domain	3.61 (3.57)	1.55 (2.22)	<0.001
GHQ- total score (/28)	20.61 (10.09)	12.21 (5.97)	<0.001
SGRQ- symptom domain (/99.99)	60.52 (24.10)	38.91 (19.27)	<0.001
SGRQ- activity domain (/99.99)	62.76 (29.52)	52.13 (25.90)	0.044
SGRQ- impact domain (/99.99)	46.36 (23.36)	26.34 (13.21)	<0.001
SGRQ- total score (/99.99)	53.69 (19.61)	34.72 (14.12)	<0.001

* GHQ denotes General Health Questionnaire, SGRQ St George's Respiratory Questionnaire

† Data presented as mean (standard deviation). Lower score represent better quality of life

Table 2. Comparison of the exercise endurance, and chronic obstructive pulmonary disease (COPD) knowledge at baseline and at the 12-week follow-up

	Baseline* (n=33)	12-week follow-up* (n=33)	P value
6-minute walking distance (m)	285 (96)	303 (98)	0.051
COPD knowledge test (/10)	6.6 (2.0)	8.8 (1.1)	<0.001

* Data presented as mean (standard deviation)

deletion of breathing exercises, which are specific to COPD.

End of intervention assessment

Primary outcome measures were the same as for COPD. Secondary outcome measures were repeat of baseline measurements to assess any change in disease knowledge, improvement in symptoms, psychological function, and muscle strength.

Results

Chronic obstructive pulmonary disease

Based on the recruitment criteria, 44 subjects with COPD participated in the Community Pulmonary Rehabilitation Programme (CPRP). Their mean age was 74 (standard deviation [SD], 7) years. After 12 weeks of CPRP, 33 subjects finished and 11 dropped out, mainly due to frequent readmissions to hospital, moving to Old Aged Homes or out of the Shatin district, transport problems, and comorbidity. Only two subjects refused exercise. Among those who completed the programme, the average attendance rate at the sessions was 78% (40%-100%). Compliance with home exercises, calculated as the number of sessions recorded in the diary divided by the prescribed number, was 77%.

Outcome evaluation

There was a statistically significant improvement in symptoms and all psychological domains (Table 1), as well as disease knowledge (Table 2). Mean exercise tolerance improved by 18 metres (6%) in the 6-minute walking test, although this was not statistically significant.

Programme evaluation

The vast majority (97%) of the subjects could follow the exercise and noted a general improvement in physical status. Most (86%) did not have problems travelling from home to the community centre. Three quarters (76%) of the participants felt that the group setting was supportive; it enabled continuous coping with their disease and were willing to re-attend any similar course next time. Over half (52%) preferred group exercise to home exercise.

In the focus group interviews regarding the disease, a major preoccupation was with finding ways to minimise the shortness of breath interfering with normal daily activities and consequential social isolation. Other comments regarding the disease included: lack of control, a desire to live longer in the event that newer, more effective treatments became available, and the expectation that the programme could improve symptoms.

Regarding the intervention programme, seven major themes emerged, relating to: acquiring knowledge, increasing exercise tolerance, encouragement to stop smoking, fewer visits to doctors or hospitals, making life happier and more meaningful, gaining a sense of accomplishment and improvement in self confidence, and psychological support.

Other general comments showed that the subjects perceived the programme as filling a service gap. However, participants wanted the group leader to demonstrate exercises by doing the moves simultaneously with the subjects at the same pace, to facilitate following all the steps. The group leaders have noted that in the group setting, participants commented on each other's health behaviours (eg smoking), and discussed the impact of their disease on family relationships.

Chronic heart failure

Thirty-seven subjects participated in the programme. The

Table 3. Evaluation of programme questionnaire

No.	Question	Disagree (%)	Ambiguous (%)	Agree (%)
1	I will attend the similar course next time	3	16	81
2	I can finish all exercises	0	9	91
3	I prefer group exercise than home exercise	28	19	53
4	I feel that my physical health is better than before	0	6	94
5	The group mates help me handle my disease	3	9	88
6	I did not have any travelling problem	0	3	97

Table 4. Baseline and 12-week follow-up data in psychosocial measures

	Baseline score* (n=37)	12-week score* (n=32)	Differences within groups* (pair=32)	P value
The Hospital Anxiety and Depression Scale†				
Anxiety	5.86 (3.84)	3.47 (3.03)	-2.41 (3.26)	<0.001
Depression	8.59 (4.67)	5.44 (3.28)	-2.97 (3.61)	<0.001
Medical Outcome Study Social Support Survey				
Tangible	67.40 (24.70)	85.94 (14.02)	16.99 (18.26)	<0.001
Affectionate	56.08 (26.55)	73.18 (26.84)	16.41 (19.68)	<0.001
Positive social interaction	46.79 (26.54)	60.94 (27.03)	13.48 (22.46)	0.002
Emotional/informational	46.96 (21.47)	59.47 (22.13)	13.28 (19.67)	0.001
The Chronic Heart Failure Questionnaire				
Dyspnoea	4.05 (0.95)	5.31 (0.92)	1.26 (0.82)	<0.001
Fatigue	4.21 (1.17)	5.01 (0.94)	0.80 (0.92)	<0.001
Emotional function	4.60 (1.39)	5.37 (0.99)	0.77 (0.85)	<0.001
Mastery	4.69 (1.20)	5.31 (0.92)	1.20 (1.03)	<0.001

* Data are presented as mean (standard deviation)

† Lower scores represent better condition

Table 5. Baseline and 12-week follow-up data in the 6-minute walking test, muscle strength test, and chronic heart failure (CHF) knowledge questionnaire

	Baseline score* (n=37)	12-week score* (n=32)	Differences within groups* (pair=32)	P value
Muscle strength test (kg)				
Quadriceps right	12.78 (4.97)	19.12 (5.28)	6.34 (5.57)	<0.001
Quadriceps left	12.88 (5.38)	18.31 (4.35)	5.43 (5.22)	<0.001
Biceps right	15.98 (6.63)	18.88 (6.20)	2.89 (4.45)	0.001
Biceps left	14.88 (5.64)	18.09 (5.45)	3.20 (3.82)	<0.001
6-minute walking test (m)	329.51 (103.18)	380.87 (90.32)	30.13 (38.93)	<0.001
CHF knowledge test (/10)	7.76 (1.69)	9.63 (0.55)	1.56 (1.39)	<0.001

* Data are presented as mean (standard deviation)

mean age of the subjects was 74 (SD, 8) years. The mean participation rate for the 12 sessions was 91% (SD, 11%). A total of 87% of the subjects completed the 12-week course. The majority gave a positive questionnaire assessment of the programme (Table 3). While there was general agreement with the beneficial nature of the programme, only about half preferred participating in a group. There was significant improvement in all psychosocial measures (Table 4), muscle strength, exercise tolerance, and disease knowledge (Table 5).

In the group interviews, discussions targeted three main themes: the reasons subjects wanted to join the programme, experience with the group programme, and the perceived benefits and effectiveness of the programme. Regarding the reasons for joining the programme, four common themes were identified: improving physical health, improving symptoms, desire for more knowledge, and a hope to reduce hospital admissions.

Seven common themes were identified in discussing the experience with the group programme: more motivation to exercise in a group; benefits of mutual support in promoting learning; enjoyment; reduced psychological burden; benefit of group sessions in behaviour modification; improved awareness of disease prevention; and increased social contact.

Regarding the benefits and effectiveness of the programme, seven themes were identified: ability to develop a regular exercise habit; improvement in symptoms; ability to modify diet; increased knowledge in disease management; psychological support; increased social contact; and prevention of hospital admissions.

Discussion

This study showed that a group programme for COPD and CHF patients is feasible in the setting of a community

centre, and was able to achieve improvement in symptoms and quality of life, with good compliance. It catered to current unmet needs, in the area of patient education, and rehabilitation group support, in an easily accessible setting. The emphasis on patient empowerment follows the Wagner model of management of chronic illness, in mobilising community resources, to enable patients to be the principal caregivers. Such models have also been widely promoted in the UK, and the US.

The main advantage of a group setting is that one group leader can cater for more than one patient at a time, whilst achieving similar results to more labour-intensive one-to-one settings. Other advantages include: feedback from patients, the exercise programme can become part of a daily social routine, knowledge can be reinforced in a group setting, and facilitation of behaviour modification such as smoking cessation. The group environment could also reduce social isolation, and anxiety/depression, possibly by improving self-efficacy or self-esteem, or through mutual support. A palliative care component may be built on this framework in future. Such a service in the setting of a community centre attached to a primary care clinic would be easily accessible, and referrals to doctors could be easily arranged. With the development of the nurse practitioner or nurse consultant, the programme can be nurse-led, or led by trained volunteers (under the supervision of a nurse, or patient leader).

Currently, in Hong Kong patients with COPD and CHF form the largest group of patients readmitted within 28 days of discharge, but it is unclear what percentage of these is avoidable. This study shows that a considerable improvement could be made in the care of these patients in the community. Addressing these unmet needs, particularly in the psychosocial category, may help reduce use of hospital services. However, improvement in quality of care of chronic diseases may not always translate into cost savings, as demonstrated for diabetes mellitus. In the case of COPD and CHF, since the intervention largely dealt with exercises, education and mutual support rather than investigations and multiple drug therapies, such a community model may result in cost savings as well as improved quality of care.

There were limitations to the study. This was essentially a pilot study where a group community intervention programme was designed and tested for feasibility and acceptability. It was not a randomised controlled trial comparing intervention versus usual management. Since there were many components in the programme, it is uncertain whether one or more of them was responsible for the good outcomes. Moreover, some of the benefits highlighted in the focus group were difficult to quantify. The number of subjects was small, and there was no information on what percentage of eligible patients would agree to join. Arguably those who participated were a select group who were already motivated. No costings were carried out, and the impact on hospital readmissions was not measured, due to the small number of subjects and the short duration of the observations. It is uncertain whether a programme of 3 months duration would have a long-lasting impact. From the exercise and psychological viewpoint, the intervention should ideally form part of the regular activity of community centres, in place of the predominantly social nature of activities. In spite of these limitations, this pilot study shows that a group community intervention programme for COPD and CHF patients is feasible and acceptable, achieves improvement in disease knowledge, symptoms and quality of life. Such a model could be developed further as an initiative in the management of chronic diseases in the community.

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References

1. Young A, Harries M Edr. Physical activity for patients: an exercise prescription. Royal College of Physicians 2001.
2. Kavanagh T, Myers MG, Baigrie RS, Mertens DJ, Sawyer P, Shephard RJ. Quality of life and cardiorespiratory function in chronic heart failure: effects of 12 months' aerobic training. Heart 1996;76:42-9.
3. Feigenbaum MS, Pollock ML. Prescription of resistance training for health and disease. Med Sci Sports Exerc 1999;31:38-45.
4. Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitations. The St. George's Respiratory Questionnaire. Am Rev Respir Dis 1992;145:1321-7.
5. Yu DSF, Lee DTF, Woo J. Establishing the validity and reliability of the Chinese versions of the Chronic Heart Failure Questionnaire and the Medical Outcome Study Social Support Survey. HSRC Report September 2002 and Annual Symposium of the 10th HK Association of Gerontology Nov 30, 2002. Available at: http://fhbgrants.netsoft.net/report/S112002fr.pdf.

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Short- and medium-term outcomes of accelerated infant growth in a Hong Kong Chinese birth cohort

Key Messages

1. In a large, population-representative, Chinese birth cohort, higher birth weight and rapid growth, particularly at 0-3 months, were associated with higher body mass index (BMI) at 7 years.
2. Boys born heavy who had grown fast had the highest BMI, but rapid growth had the largest impact in lighter-born boys.
3. Rapid growth at 0-3 months or 3-12 months was not associated with a compensatory lower risk of serious infectious morbidity.
4. The ability to grow fast may be an embodiment of good health status rather than fast growth being causally protective.

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Introduction

Cardiovascular and metabolic diseases are leading causes of death and are becoming more prevalent in Asia. These diseases are increasingly seen within a framework where causation extends over the entire life and where humans work with a limited resource base and are forced to trade-off certain life-history parameters against each other, such as a strategy which promotes survival up to reproductive age against long-term health.[1] Until very recently one of the key components of early survival was resistance to infection.

Despite two decades of intensive research, the role of foetal and infant growth in metabolic and cardiovascular diseases remains controversial. Although much attention has been focused on low birth weight as the causative factor, evidence from experiments designed to test this hypothesis have highlighted the role of nutrition-driven post-natal growth as a possible missing link in the observed relation between birth weight and adult metabolic disease. To date, observational evidence in humans suggests that higher birth weight and faster infant growth are associated with childhood obesity,[2] and hence long-term risk. Although premature cardiovascular disease is more common in men, there has been little examination into whether birth weight or infant growth (singly or jointly) has different effects depending on sex. Less attention has also been paid to other potential positive outcomes of rapid infant growth, despite a cultural preference for 'fat' babies, particularly in locations such as China. One previous study has considered the potentially complimentary survival advantage of rapid infant growth. Faster infant growth was associated with a lower risk of serious infectious morbidity and possibly mortality.[3] The impact of infant growth on health is also less well understood in developed, non-western settings, such as Hong Kong, where birth weights are typically lower and there has been a history of rapid economic development. However, such populations may act as a sentinel for other rapidly developing locations.

Identifying optimal growth trajectories potentially has major public health implications. Using data from a large population-based, prospective Chinese birth cohort, we investigated the relation between infant growth rate and two complimentary outcomes: childhood adiposity and serious infectious morbidity.

Methods

This study was conducted from October 2005 to January 2007. The 'Children of 1997' birth cohort was initiated by the Department of Community Medicine, the University of Hong Kong and the Department of Health, of the Hong Kong SAR Government.[4] The sampling frame consisted of all infants born in April and May 1997 and brought to one of any of the 47 Maternal and Child Health Centres (MCHC) for their first postnatal visit. For the index year, 92% of infants born in Hong Kong visited the MCHC, which provides free-of-charge preventive care and immunisations, at least once. The study recruited 8327 mother-infant pairs, corresponding to 88% of all births in the recruitment period.

Mothers were approached at the MCHCs a few days after delivery for recruitment and baseline data collection, and were further followed up at 3, 9

and 18 months after birth. Mothers provided information on socio-demographics (age, parental education level, employment status and type of housing), mode of delivery, parity, breastfeeding and household smoking habits via a standardised self-administered questionnaire in Chinese. In addition to this prospectively collected data, in 2005-6 record linkage was used to obtain:

- Infant growth from the MCHC hardcopy records linked by MCHC number.
- Childhood weight and height from the Student Health Services (SHS) linked by birth certificate number. The SHS provides an annual examination at no cost to all students in any Hong Kong primary or secondary school.
- Lifetime public hospital use from the Hospital Authority (HA), which provides 94% of hospital care in Hong Kong, linked by birth certificate number.

All birth cohort members have a MCHC number and 97% have a birth certificate number. When the birth certificate number was missing, matching was by name, sex and date of birth, because there are only about 55 sex-specific births per day in Hong Kong. All potential matches were checked by the research team.

Exposure: growth rates

Infant growth rate was defined as change in weight z-score at 0-3 months and 3-12 months. Weight z-score was calculated relative to the 2006 World Health Organization growth standards for the exact age on the day of measurement. The closest measurements to 3 months (within 2 to 4 month) and 12 months (within 9 to 15 months) were used.

Outcomes

Childhood adiposity

Adiposity at about age 7 years was proxied by body mass index (BMI) z-score relative to the 2000 US Centers for Disease Control and Prevention growth references for the exact age on the day of measurement. The closest measurement between 5.5 and 8.5 years was used.

Serious morbidity

Serious morbidity was proxied by number of in-patient admissions to any public hospital in Hong Kong from the age of 3 months (or 12 months for growth at 3-12 months) to 8.0 years. Admissions were classified as respiratory infections, all infections, accidents and all other causes using the International Classification of Diseases 9, Clinical Modification (ICD9-CM), of the primary discharge code. Cohort members without any record of hospital admission were assumed to have no hospital admission.

Statistical analyses

Initial analysis of the association between infant weight growth rate and childhood adiposity revealed that the associations with 0-3 month growth rate were not consistent by birth weight (P-value for interaction 0.001) or sex and birth weight (P-value for interaction 0.007). We used multivariable linear regression to assess the association of growth rate tertile, initial weight tertile (ie at start of the period) and sex with childhood BMI z-score, adjusted for gestational age (complete weeks based on date of the last menstrual period) and growth rate in the other period (as a continuous variable). Other potential confounders such as birth order, infant feeding, parental education and maternal smoking changed the estimates by less than 5% and were not included in the model.

There was no evidence that the association between either growth rate and hospital admission differed by sex or initial weight. We used multivariable negative binomial regression to calculate the relative risk of admission by growth rate tertile in each period. We adjusted for initial weight, growth rate in the other period, gestational age, sex and parental education. We additionally adjusted for disease status, ie congenital or other life-long conditions that might affect infant growth or risk of infection. Children born in private hospitals had higher socio-economic status and were more likely to use private hospitals. We also adjusted for hospital type at birth (private or public) to correct for the probable greater use of public hospitals by children from less-advantaged families. Birth order, breastfeeding and housing changed the estimates by less than 5% and were not included in the model.

The University of Hong Kong–Hong Kong Hospital Authority West Cluster Joint Institutional Review Board and the Ethics Committee of the Department of Health, Government of Hong Kong SAR approved the study.

Results

We linked 7999 of the birth cohort (96%) with the MCHC records, 7809 (94%) with the SHS records and 3746 (45%) with the HA records; not all children were expected to have a hospital admission by the age of 8 years.

Of the surviving 7832 full-term births, 7153 had weight growth rates for 0-3 months and 6874 for 3-12 months. Faster growth was more common in infants with lower birth weight, lower gestational age and more socio-economically advantaged families (Table).

Higher initial weights and higher growth rates were associated with higher childhood BMI (Fig 1). The heaviest born children with the fastest growth rate from 0-3 months had the highest BMI at 7 years, but rapid growth at 0-3 months had a greater impact on BMI in lighter-born boys.

Growth rates were not associated with admissions for respiratory infections, all infections or accidents (Fig 2). Slower growth at 0-3 months was associated with admission for other causes.

Discussion

Consistent with findings elsewhere,[2] in this understudied

Table. Baseline characteristics by growth rate tertile at 0-3 and 3-12 months for term subjects in the Hong Kong 'Children of 1997' birth cohort

Characteristics	Growth rate tertile, 0-3 months (%)				Growth rate tertile, 3-12 months (%)			
	No.	Slow (n=2483)	Medium (n=2453)	Fast (n=2217)	No.	Slow (n=2343)	Medium (n=2327)	Fast (n=2204)
Sex								
Male	3766	34.9	34.4	30.6	3633	37.7	31.9	30.4
Female	3387	34.5	34.2	31.4	3241	30.0	36.1	33.9
Birth weight								
1st tertile	2169	14.6	32.4	53.0	2101	27.2	33.6	39.2
2nd tertile	2568	32.8	37.5	29.6	2460	33.8	33.7	32.5
3rd tertile	2416	54.8	32.5	12.7	2313	40.6	34.3	24.1
Size for gestational age								
Small	702	12.0	27.6	60.4	681	26.0	31.9	42.1
Appropriate	6451	37.2	35.0	27.8	6193	35.0	34.1	31.0
Gestational age (weeks)								
37-39	4376	30.2	33.9	36.0	4203	32.5	33.1	34.4
40	1787	40.0	35.5	24.6	1722	36.1	34.3	29.6
≥41	990	45.4	34.0	20.6	949	37.3	36.4	26.3
Birth order								
1	3268	33.5	35.6	30.9	3175	29.4	34.6	36.0
2	2858	34.7	33.2	32.1	2726	38.4	33.1	28.5
3+	768	40.4	33.5	26.2	730	39.0	33.7	27.3
Mother's age (years)								
<24	882	32.0	36.3	31.8	826	35.5	31.6	32.9
25-29	2212	35.0	34.9	30.2	2119	33.6	33.8	32.6
30-34	2722	34.5	34.2	31.3	2633	34.3	34.0	31.7
35-39	1162	36.5	31.8	31.8	1123	33.8	34.9	31.3
40-44	131	36.6	37.4	26.0	130	33.1	33.9	33.1
≥45	8	25.0	12.5	62.5	8	50.0	50.0	0
Breastfeeding for 4 weeks								
No	4255	35.8	34.6	29.6	4093	33.8	33.7	32.5
Yes	2591	32.9	34.2	32.8	2508	34.6	34.3	31.1
Highest parental education attainment								
9th grade or below	2077	38.1	34.0	27.9	1914	37.8	34.0	28.3
10-11th grade	2999	35.3	34.6	30.1	2912	32.7	33.9	33.5
12th grade or above	1867	30.2	34.4	35.4	1805	32.6	33.8	33.6
Housing								
Public	3039	36.5	34.2	29.4	2895	36.0	33.3	30.7
Private	3840	33.5	34.4	32.1	3721	32.7	34.4	32.9
Birth hospital								
Public	5057	36.8	34.3	28.9	4839	34.4	34.2	31.4
Private	2049	29.5	34.3	36.2	1988	33.6	33.1	33.4
With congenital conditions								
Yes	106	49.1	33.0	17.9	101	26.7	38.6	34.7
No	7047	34.5	34.3	31.2	6773	34.2	33.8	32.0

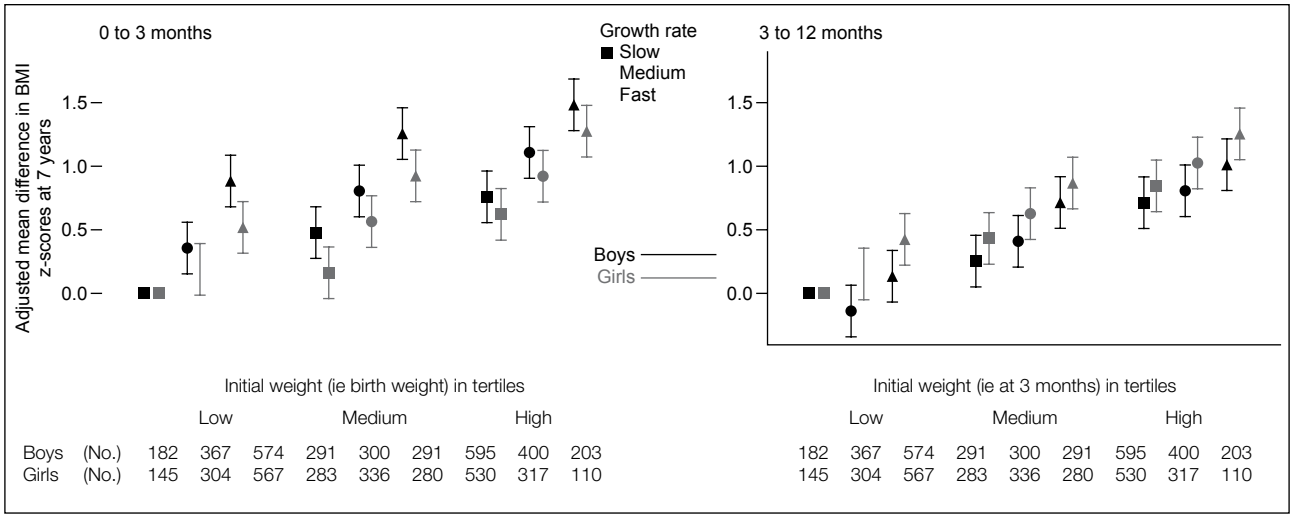

Fig 1. Adjusted* mean difference with 95% confidence intervals in body mass index (BMI) z-score at 7 years jointly by tertiles of initial weight and growth rate at 0-3 months and 3-12 months in boys and girls

* Adjusted for gestational age and growth rate in the other period

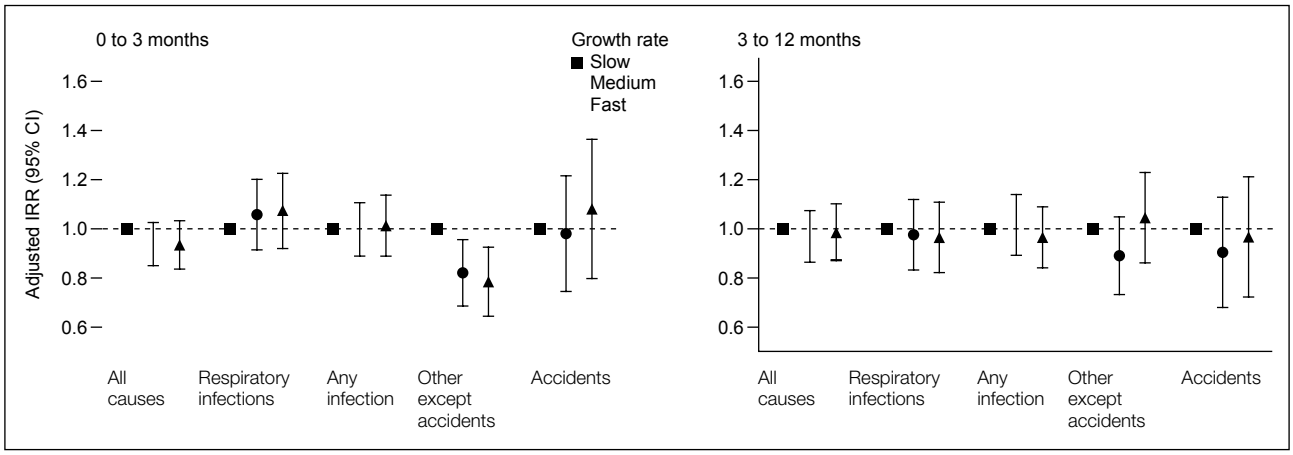

Fig 2. Adjusted* incidence rate ratio (IRR) with 95% confidence intervals (CIs) for number of hospital admissions by cause† and growth at 0-3 months and 3-12 months

* Adjusted for initial weight, growth rate in the other period, gestational age, sex, parental education, birth hospital and disease status

† Respiratory infections: ICD9-CM 033, 034.0, 381-382, 460-466, 477, 480-487 and 493; any infections: ICD9-CM 001-009, 033, 034.0, 381-382, 460-466, 477, 480-487, 493 787.91, 599.0, 780.6 and 780.3; accidents: ICD9-CM 800-999; and other except accidents: all other ICD9-CM except the above

population with scarce appropriate data, higher birth weight and faster infant weight growth were associated with higher BMIs at 7 years. Fast growth at 0-3 months was more strongly associated with higher BMI in boys born light, but not girls born light. Infants born light were also more likely to grow fast than heavier born infants. Nevertheless, the relatively small number of infants of either sex born heavy who grew fast had the highest BMI at 7 years. In contrast, rapid infant growth was not associated with a lower risk of admission for infections, but was associated with a lower risk of admission for causes other than infections or accidents. As such our study does not provide evidence that better immunity is a developmental trade-off for later metabolic risk resulting from fast infant growth. It does, however, suggest that maximal growth may not be optimal for metabolic risk.

Our study concerned a large, representative, population-based birth cohort, but had limitations. First, greater muscle mass and heavier build may explain some of the higher BMIs in high-birth-weight babies. However, muscle mass or build is unlikely to explain the differences in BMI by infant growth rate. Second, our cohort was largely fed formula milk; the impact of growth could be different in exclusively breastfed babies. Third, children with rapid infant growth may have been more likely to use private hospitals, so we cannot rule out the possibility that rapid infant growth increased the risk of hospital admission. However, we can be more confident that there was no protective effect of fast growth against serious infectious illnesses.

Most investigations into the effects of faster infant growth on metabolic risk has been from the perspective of infant growth as an outcome of, or in combination with detrimental restricted intrauterine growth. A detrimental effect of faster infant growth regardless of birth weight requires a different perspective. Disruption of hypothalamic circuit development and leptin regulation by early over-feeding could result in poorer appetite control in later life.[5] Leptin levels may also be suppressed by androgens, and hence be relevant to the differences between boys and girls.

Given the lack of benefit associated with fast growth, it is possible that traditionally valued fast infant growth (or fatness) is a marker of underlying health state, rather than a protective response to poor foetal growth. Faster growth at 0-3 months was associated with less risk of admissions for causes other than infections and accidents, of which over 50% were related to congenital anomalies, which would not always be immediately apparent.

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References

1. Van Bodegom D, May L, Meij HJ, Westendorp RG. Regulation of human life histories: the role of the inflammatory host response. Ann

NY Acad Sci 2007;1100:84-97.
2. Baird J, Fisher D, Lucas P, Kleijnen J, Roberts H, Law C. Being big or growing fast: systematic review of size and growth in infancy and later obesity. BMJ 2005;331:929.
3. Victora CG, Barros FC, Horta BL, Martorell R. Short-term benefits of catch-up growth for small-for-gestational-age infants. Int J Epidemiol 2001;30:1325-30.
4. Lam TH, Leung GM, Ho LM. The effects of environmental tobacco smoke on health services utilization in the first eighteen months of life. Pediatrics 2001;107:E91.
5. Plagemann A. Perinatal nutrition and hormone-dependent programming of food intake. Horm Res 2006;65(Suppl 3):83S-89S.

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Explaining coronary heart disease trends in Hong Kong: creation of a model for policy and planning

Key Messages

1. The largest contribution of coronary heart disease (CHD) mortality reductions was from medical treatment.
2. A smaller contribution was estimated to be due to risk factors changes.
3. Improvement of treatment uptake levels can have a substantial effect in reducing CHD mortality.

Introduction

Coronary heart disease (CHD) is the most common cause of death in developed countries. The death rate due to CHD is increasing in most developing countries and is projected to become the leading cause of death in 2020 with over 7 million deaths each year. In Hong Kong, heart disease was the second highest cause of mortality in 2005 (after cancer) and accounted for about 10% of all deaths. In 2005, the crude death rate due to CHD in Hong Kong was approximately 60 per 100 000 inhabitants. Although this CHD mortality rate is lower than that in many western countries, it is still useful to examine the impact of therapies versus changes in risk factors on CHD mortality rates, in order to predict future trends.

Many large cohort studies have already identified trends in CHD, including the World Health Organization MONICA project and the Framingham study. However, in a number of countries including China, a further modelling approach using easily available data has been used to explain trends in terms of treatment or risk factor changes.[1] We sought to apply this model to Hong Kong.

Aim

The aim of the impact model project was to relate recent CHD trends to treatments and changes in population risk factors.

Methods

This study was conducted from October 2005 to September 2006. A Microsoft Excel cell-based model was used to examine CHD mortality in Hong Kong between 1989 and 2001, as the best quality data were collected in these years. The impact model was originally created for the UK[2] and has since been applied to many other countries. The main model used in Hong Kong was that developed for the US in 2005[3] but with modifications. The model was applied to males and females aged 25 to 84 years only. The age-group of 85 years and older was not included because of limited data.

Medical and surgical treatments

Population and mortality data were obtained from the Hong Kong Census and Statistics Department and the Department of Health respectively. Numbers of discharges and deaths were obtained from the Hospital Authority clinical data. Treatment prescription rates and case fatality data were based initially on the UK model. Data on the relative risk of each treatment were obtained from published controlled trials and meta-analyses that were also used in previous impact models. The prevalence of hypertension, community angina and heart failure cases were based on local publications. Overall patient compliance and adjustments for potential overlap between CHD patient groups were based on the same assumptions as in previous impact models.

Risk factors

Changes in major risk factors may also have contributed to changes in mortality rates for CHD. The classical risk factors included in this model were blood pressure, cholesterol, body mass index, smoking and diabetes. Other factors

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Table 1. Deaths prevented or postponed by treatments in Hong Kong 1989-2001

Treatments	Deaths prevented or postponed	Minimum estimate	Maximum estimate
Acute myocardial infarction (AMI)	320	191	522
Treatments in effect 1989	-125	-40	-244
Total secondary prevention			
Secondary prevention after AMI	247	145	496
Treatments in effect 1989	-25	-8	-54
Secondary prevention after angioplasty	44	17	77
Chronic angina			
Unstable angina	10	7	18
Community angina	44	20	92
Heart failure			
Hospital heart failure	164	87	325
Community heart failure	0	-5	13
Hypertension and hyperlipidaemia treatment			
Hypertension	298	131	616
Treatments in effect in 1989	-155	-40	-401
Hyperlipidaemia	106	45	219
Total treatments			
2001	1233	637	2378
1989	-305	-88	-699
Treatments between 1989 and 2001	928	550	1678

such as physical inactivity were not included in this model since there were no reliable data. Data on risk factor prevalence were obtained from three local studies: China Light and Power study in 1990, Cardiovascular Risk Factor Prevalence (CRFP) study in 1995-96 and Population Health Survey (PHS) in 2003/2004.

Mant and Hicks correction

Multiple medications could be taken by individual CHD patients. However, the mortality reduction due to polypharmacy is unlikely to be simply the additive effect of each separate treatment. The cumulative effect was estimated by using the Mant and Hicks approach and separate relative risk reductions (RRRs) where: benefit = 1 - [(1 – RRR from treatment A) * (1 – RRR from treatment B) * … etc].

Sensitivity analysis

Sensitivity analysis was performed for each main assumption and estimate included in the model, such as uptake level, case fatality, and relative risk. Assumptions about maximum and minimum values were based on the highest or lowest values that could be obtained from internationally published studies. Otherwise, +/- 20% of the main parameters were used to generate the maximum or minimum possible for deaths prevented or postponed (DPP) for each treatment or risk factor.

Model validation

The number of DPP was estimated for each medication and cardiovascular risk in between 1989 and 2001, stratified by gender and age. This number was compared with the actual change in CHD mortality in the period, where the actual change was calculated as the number of deaths attributed to CHD in 2001 if the mortality rate stayed at the 1989 level minus the number of observed CHD deaths in 2001.

Results

Between 1989 and 2001, the actual mortality rates for CHD in Hong Kong in persons aged 25 to 84 years decreased from 79 to 76 per 100 000 in men and from 60 to 42 per 100 000 in women. The mortality rates were based on 3-year averages for the years 1988-90 and 2001-03 respectively. Using the population data and mortality rate in Hong Kong for 1989 and 2001, we estimate that there would have been 3928 CHD deaths expected in 2001 if the 1989 mortality rates had persisted, but only 2742 deaths were observed in 2001. Therefore 1186 CHD deaths (the actual fall) were prevented or postponed between 1989 and 2001.

Medical and surgical treatments

The model estimated that 1233 CHD deaths were prevented or postponed by medical and surgical treatments in 2001. After applying the Mant and Hicks correction and adjusting for treatments that were already used in 1989, there were 928 deaths estimated to be prevented or postponed by treatment between 1989 and 2001 (Table 1). Treatments for initial acute myocardial infarction (AMI), secondary prevention post AMI, as well as heart failure, hypertension and hyperlipidaemia treatments have contributed to a larger proportion of the mortality reductions; respectively estimated as 195, 221, 164 and 249 CHD deaths. Smaller contributions to DPP were estimated from secondary prevention post angioplasty (44 deaths) and treatment for chronic angina (54 deaths).

Risk factors

The changes in cardiovascular risk factors did not contribute a great deal to CHD DPP in Hong Kong between 1989 and 2001. Only 336 CHD deaths were prevented or postponed by changes in all major risk factors (Table 2). The largest contribution came from the decline in smoking prevalence, which prevented 328 CHD deaths. The decrease in mean

Table 2. Deaths prevented or postponed by risk factors in Hong Kong 1989-2001

Risk factors	Deaths prevented or postponed	Minimum estimate	Maximum estimate
Blood pressure	156	133	182
Hypertension treatments	-143	-	-
Risk factors minus treatment	13	0	156
Smoking	328	206	563
Cholesterol level	281	239	401
Cholesterol treatments	-177		
Cholesterol diet only	104	0	281
Obesity	47	34	61
Diabetes	-156	-115	-195
Risk factor total	336	125	866

diastolic blood pressure in men avoided 213 CHD deaths, while 57 more deaths were produced due to the increase in blood pressure in women. As 143 deaths were prevented by hypertension treatments, the total CHD deaths prevented by population blood pressure changes therefore decreases to 13 after adjustment. The decline in mean cholesterol levels resulted in 281 CHD DPP. However, 177 of these deaths were prevented by cholesterol treatments and only 104 by the change in cholesterol level. Decreased mean body mass index could have prevented 47 CHD deaths, 10 in men and 37 in women. Diabetes prevalence was the only risk factor that resulted in an overall increase in deaths. Increased diabetes prevalence resulted in 156 more CHD deaths.

Validation

The model estimated that a total of 1264 deaths were prevented or postponed between 1989 and 2001 compared to 1186 fewer deaths in reality. Of these, 78% of the actual reduction was attributable to treatment and 28% to risk factor changes. The model overestimated the deaths prevented or postponed and the overall model fit with actual changes in mortality is 106%—143% for men and 68% for women.

Sensitivity analyses

Sensitivity analyses showed that CHD deaths prevented or postponed were consistent among all medical treatments. The effects of all treatments together prevented 928 deaths, with a minimum of 550 and maximum 1678. All risk factor changes together prevented 336 deaths, with a minimum of 125 and maximum 866. By all risk factors changes, DPP showed a greater contribution for the maximum estimation when compared to the best estimation, which showed that DPP by risk factors may be underestimated in the model.

Discussion

As the most westernised city in China, Hong Kong has experienced a rise in CHD mortality one to two decades earlier than the remainder of China. The mortality rate for CHD in Hong Kong was still increasing in the 1970s and peaked around 1980. Although the crude death rate has remained fairly stable, age-standardised mortality has dropped substantially over the past two decades. However, the trends in mortality reduction were not similar to those in western countries. Mortality due to CHD in Hong Kong has remained low despite rapid economic growth. The mortality rate is about half of that observed in the US and UK. The influences of changes in treatments and risk factors on CHD mortality could therefore show a different effect.

Medical and surgical treatments

The advances in treatments have no doubt made a great contribution to preventing CHD mortality over the last 20 years. Some of the therapies such as statins and aspirin are very cost-effective in primary and secondary care. New surgical treatments such as angioplasty also became available over this period. Although the effect of medical treatments may be overestimated in the model, a large proportion of DPP was estimated to result from advances in treatment. However treatment uptake rates may have been relatively low for some therapies, possibly due to the cost of implementation, patients dying before arriving at hospital or the treatment not being offered. A UK study showed that improvements in uptake could make a large impact on the reduction of CHD mortality, which suggested that DPP by current treatments would be double if the uptake levels increase to 80%.[4] Thus, the effects of medical treatments in Hong Kong could be maximised if the future policies aim to improve treatment uptake of medications.

Risk factors

Only 28% of DPP was attributed to risk factor changes. The decline in smoking prevalence was attributed with the largest proportion of CHD mortality reduction among all the risk factors. A recent study proposed that the epidemic of tobacco in Hong Kong has entered an advanced period,[5] with cigarette consumption reaching a peak around 1970, 20 years later than the US, and started to decline as also observed in US. The smoking trend in Hong Kong was thus predicted to be repeating the US trend but with a two decade delay.

Mean diastolic blood pressures in Hong Kong have remained fairly stable between 1989 and 2001. However, mean systolic blood pressure was observed to increase in the same period, which is possibly due to ageing of the Hong Kong populations. Thus using diastolic blood pressure alone for calculating DPP may overestimate the real changes on mortality reduction by blood pressure.

The dietary and fat intake pattern in the local Chinese population appeared to be close to the recommended level for cardiovascular health,[6] suggesting unhealthy dietary patterns relating to fat consumption may not be a major determinant of CHD deaths. It is therefore unlikely that a large proportion of deaths from CHD could be prevented by a change of diet, at least when mediated through fat consumption. The estimated DPP associated with changes in mean cholesterol levels found over this period could be the effect of treatment. Therefore, it was assumed that the DPP due to change in cholesterol levels was mainly contributed by treatments and not lifestyle change.

Mean body mass index (BMI) for Hong Kong women has declined substantially since 1989, while the BMI for men was similar in 1989 and 2003. The decline in female BMI may be due to social pressure for a lower ideal weight for women or a consequence of including a higher proportion of middle-aged women who are employed in the data from the CRFP and PHS study, where employed middle-aged women tended to have a lower BMI than those staying at home.

Diabetes prevalence was the only risk factor that was observed to clearly increase over the study period. In the model, 156 CHD DPP were attributed to this change. The increase in diabetes among the Hong Kong population may be due to changes in lifestyle such as a decrease in physical activity.

In comparison with other countries, the low mortality rate from CHD in Hong Kong seems to be consistent with most Asian countries, except Singapore (despite having a similar level of economic development). Singapore is the only country in Asia in which the CHD mortality rate has reached the level of western countries. The difference in serum cholesterol concentrations would explain the difference in CHD mortality and could be related to the high fat intake. The Beijing model also attributed the large increase in CHD deaths in mainland China to a rise in cholesterol levels. Change in diet and other lifestyle factors from traditional Chinese to mixed modern Chinese and western patterns seems to be the main issue in changes in CHD mortality rates. It is possible that Hong Kong may experience these changes in the future if western diets continue to be popular among the younger age-groups, as indicated by the prevalence of obesity in Hong Kong children and current dietary practices.

Limitations

The impact model is highly dependent on the quality of data available. Few comprehensive studies could be found for CHD treatments and risk factors in Hong Kong, data had to be estimated using the UK and Beijing models and assumptions made in those models. Adjustments were made when possible to tailor the data for Hong Kong. Risk factor trend data were very limited in Hong Kong. The smoking trend was the only risk factor with very reliable data. For the other risk factors, only three large cohort studies were considered good enough to use in the model. This limitation of the data sources impeded delineation of trends in risk factors. Sensitivity analysis showed that the mortality reduction attributed to risk factor changes may contribute a larger proportion, as the model may have underestimated the risk factor changes in Hong Kong between 1989 and 2001 or possibly overestimated the DPP statistics due to medical treatment.

The results appear to predict more than the actual number of deaths reduced. However, the overall pattern is similar to the Beijing model, with larger contributions to mortality reductions by all medical treatments and a relatively small contribution from risk factor changes. Unlike the Beijing model, the Hong Kong model did not find an increase in deaths from risk factor changes. The Hong Kong model results fall between those for China and the US. Mortality due to CHD in Hong Kong is falling like that in the US, but locally the decline starts from a much lower peak mortality level.

Conclusions

Up to 78% of CHD mortality reduction between 1989 and 2001 was attributed to improvements in treatment while 28% was attributed to changes in population risk factors. The findings in Hong Kong were quite different to European studies, but similar to those observed in China. The CHD mortality rate in Hong Kong was already very low compared to western countries with similar levels of economic development. The model is consistent with improvements in treatment uptake and control of risk factors resulting in further CHD mortality reductions.

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References

1. Critchley J, Liu J, Zhao D, Wei W, Capewell S. Explaining the increase in coronary heart disease mortality in Beijing between 1984 and 1999. Circulation 2004;110:1236-44.
2. Capewell S, Morrison CE, McMurray JJ. Contribution of modern cardiovascular treatment and risk factor changes to the decline in coronary heart disease mortality in Scotland between 1975 and 1994. Heart 1999;81:380-6.
3. Ford ES, Ajani UA, Croft JB, et al. Explaining the decrease in U.S. deaths from coronary disease, 1980-2000. N Engl J Med 2007;356:2388-98.
4. Capewell S, Pell JP, Morrison C, McMurray J. Increasing the impact of cardiological treatments. How best to reduce deaths. Eur Heart J 1999;20:1386-92.
5. Lam TH, Ho SY, Hedley AJ, Mak KH, Peto R. Mortality and smoking in Hong Kong: casecontrol study of all adult deaths in 1998. BMJ 2001;323:361.
6. Woo J, Leung SS, Ho SC, Sham A, Lam TH, Janus ED. Dietary practices and lipid intake in relation to plasma lipid profile in Hong Kong Chinese. Eur J Clin Nutr 1997;51:467-71.

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Rapid detection of food-borne pathogens in clinical specimens, food and environmental samples

Key Messages

1. PCR and RT-PCR methods for 5-hour and 3-hour detection, respectively, of salmonellae and *Vibrio cholerae* in stool, food and environmental water samples have been developed.
2. Such methods can be used in routine laboratories for rapid detection of salmonellae and *V cholerae* and are essential for infection control purposes.

Introduction

Salmonella species are the most common bacterial cause of diarrhoea and *Vibrio cholerae* occasionally cause diarrhoeal outbreaks in Hong Kong. Salmonellae can also cause invasive diseases such as septicaemia or meningitis especially in young children. Gastroenteritis caused by both types of organisms is transmitted via contaminated foods and infected persons.

Laboratory diagnosis of salmonellosis and cholera and location of the infectious source in foods depend on traditional methods of culture and identification. These methods take at least 2 days to yield results thus delaying the institution of prompt prevention and control measures.

For these reasons we aimed to develop rapid molecular biological methods for the detection of salmonellae and *V cholerae* in clinical, food and water samples. We also aimed to optimise these methods for routine use in a diagnostic and public health laboratory and to use these methods for detecting organisms prospectively in various clinical, food and environmental samples taken for surveillance purposes.

Methods

All surviving salmonellae isolated during 2000-2003 were tested for the presence of genes specific to *Salmonella* species by a multiplex polymerase chain reaction (PCR).[1] The detection limit of the PCR was determined using 10-fold serial dilutions of overnight colonies of a standard strain of *S choleraesuis* (ATCC 13076). The specificity was determined by testing a variety of bacterial organisms other than salmonellae. All stool specimens (including rectal swabs) received for bacterial culture by our laboratory during the period May 2005 to August 2006, a variety of food items, and water samples collected from three different beaches were cultured for stool pathogens using standard procedures and subjected to PCR for detection of salmonellae. Real-time PCR (RT-PCR)[2] was also used to detect salmonellae in pure culture, stool samples, foods and water.

All surviving *V cholerae* in our culture collection (since 1983) were subjected to multiplex and hexaplex PCR.[3,4] The detection limit and specificity of the PCR were tested as described above. Since no *V cholerae* were cultured from stool specimens during the period of study, nor from the same food items and water samples used above, 0.5 μL of DNA extracted from each of the serial 10-fold dilutions of *V cholerae* was added before performing the PCR. Also RT-PCR[5] was used to detect vibrios in pure culture, stool samples, foods and water.

Results

All 410 isolates of 58 serotypes of salmonellae isolated during 2000-2003 were positive on PCR for the *invA* gene while only 15% were positive for the *spvC* gene (Table 1). *S enteritidis*, *S typhimurium* and *S derby* were the most common salmonellae tested, each with 10% or more isolates. More than 90% of *S enteritidis*, only 22% of *S typhimurium* but none of the *S derby* isolates had both the *spvC* and *invA* genes.

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Table 1. *Salmonella* serotypes tested for presence of *spvC* and *invA* genes

Salmonella serotype	Total tested		*spvC- invA+*		*spvC+ invA+*	
	No.	%	No.	%	No.	%
S enteritidis	52	13	4	8	48	92
S typhimurium	46	11	36	78	10	22
S derby	40	10	40	100	0	0
S stanley	35	9	35	100	0	0
S rissen	28	7	28	100	0	0
S saintpaul	22	5	22	100	0	0
S virchow	22	5	22	100	0	0
S typhi	12	3	12	100	0	0
S heidelberg	11	3	10	91	1	9
S hadar	10	2	10	100	0	0
S weltevreden	10	2	10	100	0	0
S anatum	8	2	8	100	0	0
S give	8	2	8	100	0	0
S agona	7	2	7	100	0	0
S infantis	7	2	7	100	0	0
S paratyphi A	7	2	7	100	0	0
S newport	6	1	6	100	0	0
S reading	5	1	5	100	0	0
S thompson	5	1	5	100	0	0
S bareily	4	1	4	100	0	0
S braenderup	4	1	4	100	0	0
S london	4	1	4	100	0	0
S bovis-morbificans	2	0	1	50	1	50
Others*	52	13	52	100	0	0
Total	410	100	350	85	60	15

* Includes three strains each of *S bardo*, *S haardt*, *S manhattan*, *S nchanga*, *S niensteden* and *S panama*, two strains each of *S blockley*, *S galiena*, *S indiana*, *S krefeld*, *S lomita*, *S mbandaka*, *S tenessee* and *S wandsworth*, and one strain each of *S aberdeen*, *S bonn*, *S eppendorf*, *S giza*, *S hartford*, *S kentucky*, *S lagos*, *S lockleaze*, *S montevideo*, *S muenchen*, *S muenster*, *S oslo*, *S potsdam*, *S seegefeld*, *S shubra*, *S singapore*, *S sinstorf*, *S texas*, *S uganda*, *S uppsala* and *S zanzibar*, all of which had the *invA* gene only

The detection limit for *invA* using PCR was $9.6x10^2$ cfu/mL while that for *spvC* was $9.6x10^3$ cfu/mL. One PCR cost approximately HK$6 while culture and identification of one salmonella strain from one stool specimen cost approximately HK$38.

The following 32 organisms were tested for *spvC* and *invA* and none was positive: *Aeromonas hydrophila*, *Citrobacter* sp, *C freundii*, *C koseri*, *Campylobacter jejuni*, *Enterobacter* sp, *E aerogenes*, *E cloacae*, *Escherichia coli*, *Helicobacter pylori*, *Klebsiella* sp, *K oxytoca*, *K pneumoniae*, *Plesiomonas shigelloides*, *Pseudomonas aeruginosa*, *P putida*, *Proteus mirabilis*, *P vulgaris*, *Providencia stuartii*, *Shigella boydii*, *S dysenteriae*, *S flexneri*, *S sonnei*, *Serratia sp*, *S marcescens*, *Vibrio cholerae*, *V parahaemolyticus*, *Bacillus cereus*, *Enterococcus sp, E faecalis*, *E faecium* and *Staphylococcus aureus*.

More than 11 000 stool specimens were received for bacterial culture from May 2005 to August 2006. The stool culture positive rate for salmonella ranged from 0.9% to 4.9%, averaging 3.1%. Five different sample preparation and PCR conditions were tried and optimised on >3000 stool specimens. The method showing highest sensitivity was used on the remaining specimens, giving an overall sensitivity of 81%. The detection limit was $4.8x10^4$ cfu/mL and $9.6x10^4$ cfu/mL for *invA* and *spvC*, respectively. Specimens that were negative for salmonellae by PCR but positive by culture had very low cell counts and were loose or soft stools. The PCR on stools took approximately 5 hours to produce a result.

All food and water samples (Table 2) were negative for salmonellae. DNA from different cfu/mL of salmonellae was seeded into these samples to test the detection limit of the PCR method. It ranged from $9.6x10^3$ cfu/mL to $9.6x10^6$ cfu/mL.

RT-PCR was performed on pure cultures of 43 of the 58 salmonella serotypes (Table 1) and four each of serogroups B, C, D and E (serotypes could not be determined) and all gave a positive reaction. It was also performed on 94 salmonella culture-positive stool samples (50 PCR positive,

Table 2. Detection limit for *invA* and *spvC* in food items and environmental water samples

Source of sample	Detection limit ($x10^3$ cfu/mL) for*									
	invA					*spvC*				
	9600	960	96	48	9.6	9600	960	96	48	9.6
Food										
Raw oyster (Irish)	+	-	-	-	-	+	-	-	-	-
Ice-cream	+	+	-	-	-	+	+	-	-	-
Fresh pork, intestine (chicken), raw oyster (Australian)	+	+	+	-	-	+	+	-	-	-
Chocolate milk, juice	+	+	+	+	-	+	+	-	-	-
Small intestine (pig)	+	+	+	+	+	+	+	-	-	-
Sweet bean curd, roasted duck, ham, salmon sashimi	NT	NT	+	+	-	NT	NT	+	-	-
Drinking yoghurt, colon and kidney of pig, barbecued pork, bivalve, salami, cooked simesaba sashimi	NT	NT	+	+	-	NT	NT	+	+	-
Roasted pork	NT	NT	+	+	+	NT	NT	+	+	+
Water										
Stanley Main Beach	NT	NT	+	+	+	NT	NT	+	+	+
Turtle Cove	NT	NT	+	+	-	NT	NT	+	+	-
Wong Shek	NT	NT	+	+	+	NT	NT	+	+	+

* + denotes detected, - not detected, and NT not tested

Table 3. Detection limit for *rfb* and *ctxA* genes by polymerase chain reaction of food and environmental water samples

Source of sample	Detection limit (x10³ cfu/mL) for*							
	O1				O139			
	80		8		120		12	
	rfb	*ctxA*	*rfb*	*ctxA*	*rfb*	*ctxA*	*Rfb*	*ctxA*
Food								
Sweet bean curd, drinking yoghurt, chocolate milk, juice, fresh pork, intestine (chicken), colon and kidney of pig, roasted pork, barbecued pork, roasted duck, ham, salami, cooked simesaba sashimi	+	+	+	+	+	+	+	+
Bivalve	+	+	+	-	+	+	+	+
Ice-cream	+	+	+	-	+	+	-	-
Salmon sashimi	+	+	-	-	+	+	+	+
Raw oyster (Irish and Australian)	+	+	-	-	+	+	-	-
Water								
Stanley Main Beach	+	+	+	+	+	+	+	+
Turtle Cove	+	+	+	+	+	+	+	+
Wong Shek	+	+	+	+	+	+	+	+

* + denotes detected, and - not detected

44 PCR negative). All 50 PCR positive and 33 PCR negative samples were RT-PCR positive giving a total positive rate of 88% in contrast to 53% on PCR. The detection limit was $4.0x10^2$ cfu/mL. Nevertheless, 11 (12%) were both PCR and RT-PCR negative. The RT-PCR for one stool specimen took approximately 3 hours and cost approximately HK$8.

The RT-PCR on all food and water samples tested had a detection limit of $9.6x10^2$ to $9.6x10^3$ cfu/mL.

A total of 24 isolates of *V cholerae* were subjected to multiplex and hexaplex PCR and all gave positive results. The detection limit in pure culture was 80 to $1.2x10^2$ cfu/mL but was $8x10^3$ to $1.2x10^4$ cfu/mL for stool samples. Nevertheless, since only multiplex PCR could differentiate between O1 and O139, we propose that multiplex PCR be used to detect *V cholerae*.

40 organisms were tested using PCR and all gave negative results: *V alginolyticus*, *V campbelli*, *V fluvalis*, *V furnissii*, *V harveyi*, *V marinus*, *V parahaemolyticus*, *V pelagius*, *V splendidus*, *A hydrophila*, *Citrobacter* sp, *C freundii*, *C koseri*, *Enterobacter* sp, *E aerogenes*, *E cloacae*, *E coli*, *H pylori*, *Klebsiella* sp, *K pneumoniae*, *K oxytoca*, *P aeruginosa*, *P putida*, *P mirabilis*, *P vulgaris*, *P stuartii*, *P shigelloides*, *S boydii*, *S dysenteriae*, *S flexneri*, *S sonnei*, *S typhimurium, Serratia* sp, *S marcescens*, *B cereus*, *Corynebacterium jeikeium*, *Enterococcus* sp, *E faecalis*, *E faecium* and *S aureus*.

Approximately 200 stool samples were seeded with *V cholerae* O1 or O139 DNA then subjected to PCR; 99% were positive for O1 *V cholerae* and 85% for O139. The detection limit was $8x10^3$ cfu/mL for O1 and $1.2x10^4$ cfu/mL for O139. The food and water samples lacking *V cholerae*, were also seeded with *V cholerae* O1 or O139 DNA and subjected to PCR. The detection limit of O1 varied from $8x10^3$ to $8x10^4$ cfu/mL and that of O139 from $1.2x10^4$ to $1.2x10^5$ cfu/mL (Table 3).

The 24 *V cholerae* strains tested by PCR were also tested by RT-PCR. All were positive. All three PCR-positive stool samples and 30 of 33 PCR-negative samples were RT-PCR positive when $8x10^2$ cfu/mL were present. The remaining three were RT-PCR positive when $8x10^3$ cfu/mL were present. The RT-PCR performed on all food and water samples had a detection limit of $8x10^2$ to $8x10^3$ cfu/mL.

Discussion

None of the 32 non-salmonella bacterial species tested were positive for the *invA* gene (present on the chromosome), but all the salmonella serotypes were, indicating that detection of this gene is a sensitive and specific method for salmonellae. Only a small percentage of salmonellae (15%) were positive for the *spvC* gene, however, *spvC* is present on a virulence plasmid which may not be present in all strains.

Our PCR method could detect salmonellae at levels as low as $9.6x10^2$ cfu/mL in pure culture, although at least $4.8x10^4$ cfu/mL was required for its detection in stools. As PCR inhibitors are often present in stools, the detection limit is expected to be higher.

Although our PCR method achieved only 81% sensitivity, it yields same-day results. We did not incubate our specimens to increase the number of organisms, thus those containing few organisms gave negative results. Other studies with higher sensitivity rates achieve these by incubating specimens but results are not available until the next day. Specimens containing large numbers of organisms are highly infectious compared with those containing smaller numbers so a same-day result is desirable. To avoid missing salmonella-positive stools that are PCR-negative due to inhibitors or other unknown factors, we propose that the same specimen be subjected to both PCR and culture. Polymerase chain reaction provides a rapid result while the culture may confirm any false-negatives.

The PCR detection limit for salmonellae in food and environmental water was comparable with or higher than that in stool samples. It is difficult to explain why detectable salmonella numbers had to be higher in fresh foods than processed foods, as additives contained in the latter should inhibit the amplification reaction. Since such high salmonella counts are unlikely to be present in food or water samples, we propose that a culture and PCR be performed on the same sample.

The RT-PCR method gave a higher positive rate than the PCR method, indicating that the former is a more sensitive method. Both methods give same-day results and performing RT-PCR is only slightly more expensive than a PCR. Both are much cheaper than culture and identification.

Since the detection limit for *V cholerae* in stools was 10- to 100-fold lower than that for salmonellae, our method is sufficiently sensitive for detecting *V cholerae*. Patients with cholera usually excrete the organism in numbers well above the detection limit. Although both the hexaplex PCR and multiplex PCR can detect *V cholerae*, only the multiplex PCR can differentiate O1 from O139. Therefore the multiplex PCR, which gave positive rates of 99% and 85% for O1 and O139 respectively, should be used to detect *V cholerae*.

Although we had no stool specimens positive for *V cholerae*, our results with virtual *V cholerae*–containing stool samples indicated that our method was very sensitive. As with salmonellae, we propose that concomitant cultures and multiplex PCR be performed on specimens to avoid false-negative PCR results. Polymerase chain reaction was very specific for detecting *V cholerae* as none of 40 other organisms tested (mostly normal bowel commensals) were positive.

The *V cholerae* detection limit in food and water samples was similar to or higher than that in stools, indicating the presence of other substances that may affect the amplification procedure.

Using RT-PCR, we could detect *V cholerae* at a lower limit than when using PCR. Since it is not much more expensive to perform, it is better to use RT-PCR for detecting both *V cholerae* and salmonellae in stool or food samples. Nevertheless, RT-PCR requires special, costly, equipment that may be too expensive for some diagnostic laboratories while PCR requires only a simple thermal cycler that is currently reasonably inexpensive. Hence PCR may be better for diagnostic laboratories switching to molecular detection of stool pathogens.

We have developed specific methods using PCR and RT-PCR for same-day detection of salmonellae and *V cholerae* in stool, food and water samples. These methods gave ≥80% detection rates with the remaining ≤20% being undetected due to their very low organism counts.

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References

1. Chiu CH, Ou JT. Rapid identification of *Salmonella* serovars in feces by specific detection of virulence genes, *invA* and *spvC*, by an enrichment broth culture-multiplex PCR combination assay. J Clin Microbiol 1996;34:2619-22.
2. Cheung PY, Chan CW, Wong W, Cheung TL, Kam KM. Evaluation of two real-time polymerase chain reaction pathogen detection kits for *Salmonella* spp. in food. Lett Appl Microbiol 2004;39:509-15.
3. Hoshino K, Yamasaki S, Mukhopadhyay AK, et al. Development and evaluation of a multiplex PCR assay for rapid detection of toxigenic *Vibrio cholerae* O1 and O139. FEMS Immunol Med Microbiol 1998;20:201-7.
4. Singh DV, Isac SR, Colwell RR. Development of a hexaplex PCR assay for rapid detection of virulence and regulatory genes in *Vibrio cholerae* and *Vibrio mimicus*. J Clin Microbiol 2002;40:4321-4.
5. Lyon WJ. TaqMan PCR for detection of *Vibrio cholerae* O1, O139, non-O1, and non-O139 in pure cultures, raw oysters, and synthetic seawater. Appl Environ Microbiol 2001;67:4685-93.

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Environmental surveillance for *Laribacter hongkongensis*, a diarrhoeal pathogen discovered in Hong Kong

Key Messages

1. *Laribacter hongkongensis* was isolated from the midguts and hindguts of 86 (24%) of 360 freshwater fish from retail markets, including grass carp (60%), bighead carp (53%), mud carp (25%), and large-mouth bass (5%).
2. This study is the first to demonstrate the presence of *L hongkongensis* in natural water environments, with the bacterium being isolated from the waters of six reservoirs, with higher recovery rates in summer and during days of higher water and ambient temperatures.
3. Molecular typing using pulsed-field gel electrophoresis revealed a heterogenous population of *L hongkongensis* in both the freshwater fish and drinking water reservoir isolates, suggesting that the bacterium is endemic in our freshwater environments.
4. Since freshwater fish are common food items for our population, the general public should be educated on the proper preparation and thorough cooking of freshwater fish before consumption to avoid *L hongkongensis*–associated gastroenteritis.
5. Although it is unlikely that treated drinking water is a significant source of *L hongkongensis*–associated gastroenteritis, it is important to be aware of the possibility of other contaminated water as a source of human infection.

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Introduction

Laribacter hongkongensis, a novel genus and species, was first isolated in Hong Kong in 2001 from the blood and empyaema pus of a 54-year-old Chinese man with alcoholic cirrhosis, bacteraemia and empyaema.[1] Phenotypically, it is a facultative anaerobic, motile, non-sporulating, urease-positive, Gram-negative, S-shaped bacillus. By phylogenetic analysis using 16S rRNA gene sequences, *L hongkongensis* belongs to the *Neisseriaceae* family of the β-subclass of *Proteobacteria*. It was subsequently discovered in the stools of six patients with community-acquired gastroenteritis in Hong Kong and Switzerland.[2] Using cefoperazone MacConkey agar as the selective medium,[3] we confirmed that *L hongkongensis* is associated with community-acquired gastroenteritis and traveller's diarrhoea.[4] Furthermore, it was confirmed that freshwater fish are a reservoir for *L hongkongensis*.[4] The isolation of *L hongkongensis* from patients who either resided in or had histories of recent travel to Asia, Europe, America, and Africa implies that the bacterium is of global importance.

Methods

This study was conducted from December 2004 to November 2006.

Study design

To determine the prevalence of *L hongkongensis* in the freshwater fish population in our locality, we carried out a territory-wide animal and environmental surveillance study of commonly consumed freshwater fish purchased from local retail markets. We also investigated the presence of *L hongkongensis* in samples from drinking water reservoirs in Hong Kong. Freshwater fish from these reservoirs were also sampled. All *L hongkongensis* isolates were typed by pulsed-field gel electrophoresis (PFGE) and the patterns analysed and compared.

Sample size

A total of 360 freshwater fish from the six different species commonly purchased for cooking in Hong Kong were obtained from retail food markets (six fish per species per market) in different districts of Hong Kong. The fish were grass carp (*Ctenoharyngodon idellus*), bighead carp (*Aristichthys nobilis*), mud carp (*Cirrhina molitorella*), large-mouth bass (*Micropterus salmoides*), Chinese perch (*Siniperca chuatsi*) and tilapia (*Oreochromis mossambicus*).[5,6]

Water samples were collected from 10 drinking water reservoirs located in different regions of Hong Kong, namely Pok Fu Lam, Tai Tam, Aberdeen, High Island, Shing Mun, Kowloon, Shek Lei Pui, Tai Lam Chung, Plover Cove and Shek Pik reservoirs. Samples were collected every 3 months over a 1-year period (October 2003 to September 2004). Where possible, fish were also obtained from these reservoirs during the non-spawning season (October 2003 to March 2004).[7]

Sampling methods and analysis

Samples were obtained from the midguts and hindguts of the fish using sterile cotton wool swabs. All samples were plated onto cefoperazone MacConkey agar and incubated in aerobic conditions at 37°C for 48 h. Water samples (in

volumes of 2000 mL) were collected in pre-sterilised 1000 mL bottles submerged to a depth of 50 cm. Detection of *L hongkongensis* was performed using the membrane filtration technique. Volumes of 100 mL were filtered through membrane filters which were incubated on CMA at 37°C for 48 h. A total of 2000 mL water was tested from each reservoir each time and the colony counts were expressed as colonies/L. All suspected bacterial isolates were identified phenotypically using standard biochemical methods. Isolates suspected to be *L hongkongensis* were subject to 16S ribosomal RNA gene sequencing.[1] Pulsed-field gel electrophoresis was performed using bacterial plugs digested with *Spe*I in 0.5× TBE buffer and the CHEF Mapper XA System (Bio-Rad Laboratories, Hercules [CA], US). Digital images were stored electronically as TIFF files and analysed visually and with GelCompar II (version 3.0; Applied Maths, Kortrijk, Belgium).

Results

Isolation of Laribacter hongkongensis from retail freshwater fish

Laribacter hongkongensis was isolated from the midguts and hindguts of 86 (24%) of 360 freshwater fish. It was isolated from 36 (60%) of 60 grass carp, 32 (53%) of 60 bighead carp, 15 (25%) of 60 mud carp, and three (5%) of 60 large-mouth bass. Overall, 67 different PFGE patterns were found in the 86 *L hongkongensis* isolates from freshwater fish.[5]

Isolation of Laribacter hongkongensis from water of reservoirs

Laribacter hongkongensis was isolated from the waters of six (60%) of 10 drinking water reservoirs, with numbers ranging from 1 to 12 cfu/L. The numbers ranged from 0 to 1 cfu/L in autumn and winter, 0 to 9 cfu/L in spring and 0 to 12 cfu/L in summer. There was a significant difference in the mean numbers of *L hongkongensis* in reservoir waters in different seasons (P=0.046). Higher numbers were observed in summer than in autumn (P≤0.04) and winter (P=0.046). There was a positive correlation between the numbers of *L hongkongensis* and the water temperature (Pearson correlation 0.379, P=0.016), and between numbers and ambient temperature (Pearson correlation 0.39, P=0.013). A total of 27 freshwater fish from 10 species were collected from six reservoirs, including 15 during the autumn and 12 during the winter. *Laribacter hongkongensis* was recovered from the intestines of two of the 27 fish, a Goldfish (GC2) and a Nile Tilapia (NT4), collected in autumn from Pok Fu Lam reservoir and Plover Cove reservoirs respectively. Overall, 35 different PFGE patterns were identified among the 59 isolates of *L hongkongensis* recovered from water and the two isolates from freshwater fish.[7]

Discussion

This report demonstrates the existence of *L hongkongensis* in natural water environments. Its presence in the water samples of six of the 10 sampled reservoirs suggests that the bacterium is prevalent in the drinking water reservoirs of Hong Kong, although the significance of *L hongkongensis* in freshwater environments is yet to be determined. Drinking water is subjected to purification procedures in advanced treatment plants before being distributed to residents. The risk of acquiring *L hongkongensis* from drinking tap water is therefore likely to be low, but the significance of other water-borne sources of *L hongkongensis* in humans warrants further investigation.

The presence of *L hongkongensis* in drinking water reservoirs is also likely to be related to the freshwater fish reared in the reservoirs. *Laribacter hongkongensis* was not detected in the water from the three reservoirs where water from Dongjiang is stored. This suggests that water from Dongjiang is probably not an important source of *L hongkongensis* in the reservoirs. The waters collected from local streams are mainly from rainfall and there are no notable contamination sources in the catchment areas. Therefore, the most likely source of the bacterium is the freshwater fish in the reservoirs.

Freshwater fish, especially carp, is probably the major reservoir for human infections with *L hongkongensis*. Molecular typing showed that a heterogeneous population of *L hongkongensis* was present in both freshwater fish and natural waters in Hong Kong, suggesting that the bacterium is endemic in our locality. It is probably also endemic in southern China, where over half of the patients reported in our previous study had recently travelled prior to developing *Laribacter* gastroenteritis. Since *L hongkongensis–associated* gastroenteritis is associated with eating fish and the bacterium can be found in diverse freshwater fish species; caution should be taken when handling and cooking any freshwater fish to prevent infections associated with *L hongkongensis* and other freshwater fish related pathogens.

Acknowledgement

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References

1. Yuen KY, Woo PC, Teng JL, Leung KW, Wong MK, Lau SK. *Laribacter hongkongensis* gen. nov., sp. nov., a novel gram-negative bacterium isolated from a cirrhotic patient with bacteremia and empyema. J Clin Microbiol 2001;39:4227-32.
2. Woo PC, Kuhnert P, Burnens AP, et al. *Laribacter hongkongensis*: a potential cause of infectious diarrhea. Diagn Microbiol Infect Dis 2003;47:551-6.
3. Lau SK, Woo PC, Hui WT, et al. Use of cefoperazone MacConkey agar for selective isolation of *Laribacter hongkongensis*. J Clin Microbiol 2003;41:4839-41.
4. Woo PC, Lau SK, Teng JL, et al. Association of *Laribacter hongkongensis* in community-acquired gastroenteritis with travel and eating fish: a multicentre case-control study. Lancet

2004;363:1941-7.
5. Teng JL, Woo PC, Ma SS, et al. Ecoepidemiology of *Laribacter hongkongensis*, a novel bacterium associated with gastroenteritis. J Clin Microbiol 2005;43:919-22.
6. Lau SK, Woo PC, Fan RY, Lee RC, Teng JL, Yuen KY. Seasonal and tissue distribution of *Laribacter hongkongensis*, a novel bacterium associated with gastroenteritis, in retail freshwater fish in Hong Kong. Int J Food Microbiol 2007;113:62-6.
7. Lau SK, Woo PC, Fan RY, et al. Isolation of *Laribacter hongkongensis*, a novel bacterium associated with gastroenteritis, from drinking

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Mouse studies of SARS coronavirus–specific immune responses to recombinant replication-defective adenovirus expressing SARS coronavirus N protein

Key Messages

1. A recombinant adenovirus encoding SARS coronavirus (SARS-CoV) nucleocapsid protein (rAd-N) was constructed.
2. The ability of the rAd-N to induce anti–SARS-CoV N antibody production and cellular immune responses was evaluated in an HLA-A2.1/Kb transgenic mouse model.

Introduction

This project aimed to test a new vaccine technology platform using an adenovirus vector to produce a recombinant vaccine against severe acute respiratory syndrome (SARS) infection. A recombinant adenovirus (rAd) encoding SARS coronavirus (SARS-CoV) nucleocapsid protein (rAd-N) was constructed, and the ability of the rAd-N to induce anti–SARS-CoV N antibody production and cellular immune responses was evaluated. Evidence from human gene therapy and vaccine trials has established a good safety record for Ad5- and Ad2-based vectors. Both vectors are safe when administered by intramuscular and intradermal injection, as well as via intranasal inhalation, which may be a particularly important means of inducing mucosal immunity to respiratory infectious diseases such as SARS. Recently, exciting results from human immunodeficiency virus (HIV) vaccine research into an adenovirus type 5 (Ad 5) vector have emerged from primate and human clinical trials.

Materials and methods

This study was conducted from July 2004 to June 2006. SARS-CoV N protein was expressed and purified from bacteria. The DNA fragment encoding the SARS-CoV N protein was generated by a polymerase chain reaction using the following primers: AdN-5' primer (GGAATTCCATATCTCTGATAATGGACCCCAATC) and AdN-3' primer (CATGGGATCCGCCTGAGTTGAATCAGCAG) and cloned into the pET22b(+) vector (Novagen) in fusion with 10 histidines. The sequence of the SARS-CoV N was confirmed, and the resulting plasmid was subsequently transformed into BL21-CodonPlus (DE3)-RIL (Stratagene). Expression of the SARS-CoV N protein in bacterial cells was induced using 0.4 mM IPTG (Calbiochem) for 4 hours at 30ºC. Soluble N protein was purified by His-Bind Kit (Novagen). Anti-N protein monoclonal antibodies were produced using 6-week-old female Balb/c mice (Animal and Plant Care Facility, HKUST). A hybridoma cell line yielding anti-N antibodies was produced and kept in liquid nitrogen for long-term storage.

QBI-HEK 293 cells (Q-biogene) are a strong plastic-attached sub-clone of HEK-293 cells (primary human kidney) containing the E1A and E1B Ad 5 viral genes for the generation and titration of rAd. Human cervix epithelial adenocarcinoma GH329 cells (ATCC) were stably transfected with plasmid carrying Ad 5 E1a and E1b open reading frames and part of the *pIX* gene. The cells were used for propagation of the rAd, and were maintained in culture according to the supplier's specifications.

The rAd-N encoding the SARS-CoV nucleocapsid protein was generated. Briefly, a full-length SARS-CoV N gene was inserted into the Transpose-Ad transfer vector, pCR259 (Q-biogene), to generate pCR-AdN. Integrity of the pCR-AdN was checked using restriction endonuclease cleavage and DNA sequencing. pCR-AdN was subsequently transformed into HighQ-1 Transpose-Ad 294 bacteria to generate a plasmid, p294-AdN. p294-AdN was linearised and transfected into QBI HEK-293 cells for the generation of rAd, rAd-N. rAd-N and rAd-lacZ (provided

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by the supplier) were propagated in GH329 cells and purified by an Adeno-X virus purification kit (BD Bioscience). Titres (Tissue culture infectious dose, $TCID_{50}$) of the rAds were determined by their end-point dilutions. For infection, HEK 293 cells were given a multiplicity of infection (MOI=20 for 24 h) with rAd-N and rAd-lacZ. Expression of the SARS-CoV N protein was checked using a Western blot analysis of the infected cell lysate, using a monoclonal antibody against SARS-CoV N protein as described above.

Vaccination of the HLA-A2.1/Kb transgenic mice: HLA-A2.1/Kb transgenic mice (Mutant Mouse Regional Resource Centers, US) were bred and maintained at the Animal Care Centre (HKUST). All procedures were performed in accordance with the international guidelines for experimental animals. Three groups (five mice per group) of 6-to-8-week-old female HLA-A2.1/Kb transgenic mice were used for vaccination via intraperitoneal injection according to the following scheme: group 1 was immunised with PBS using a dosage of 0.1 mL/mouse; group 2 was immunised with 1×10^8 $TCID_{50}$ rAd-N using a dosage of 0.1 mL/mouse; group 3 was immunised with 1×10^8 $TCID_{50}$ rAd-LacZ using a dosage of 0.1 mL/mouse. The mice were killed 3 weeks after immunisation, and examined for both humoral and cellular immune responses using the enzyme-linked immunosorbent assay (ELISA) and IFN-γ ELISPOT assay, respectively.

Results and discussion

SARS-CoV N protein was expressed in BL21-CodonPlus (DE3)-RIL after IPTG induction. Purified soluble N protein was checked using SDS-PAGE. After IPTG induction, an additional band of 49kDa was found in the total cell lysate, which is consistent with the predicted size of the His-tagged recombinant N protein. After purification, a protein preparation consisting of a single band of the ~49kDa recombinant N protein was obtained (Fig 1a). Expression of the SARS-CoV N protein from the rAd was demonstrated by infecting the HEK 293 cells with rAd-N and rAd-lacZ at MOI=20 for 24 h. After viral infection, cell lysate was prepared and the expression of the SARS-CoV N protein was confirmed by Western blot. A specific band of the N protein was clearly observed in the rAd-N infected HEK-293 cells by using monoclonal antibodies against the SARS-CoV N protein, whereas no N protein band was found in the parental cells and the cells infected by the rAd-lacZ (Fig 1b). The result indicated that infection of the rAd-N is sufficient to mediate the SARS-CoV protein expression.

To assess the humoral immune response upon vaccination with rAd-N, sera from mice immunised with either 1×10^8 $TCID_{50}$ rAd-N, rAd-lacZ or PBS, were collected and antibodies against SARS-CoV N protein were measured by ELISA. The result revealed a significant increase in antibody level against the SARS-CoV N protein, suggesting that immunisation of rAd-N was able to induce the production of anti-N protein antibodies in vivo (Fig 2).

The cellular immune response is a major component

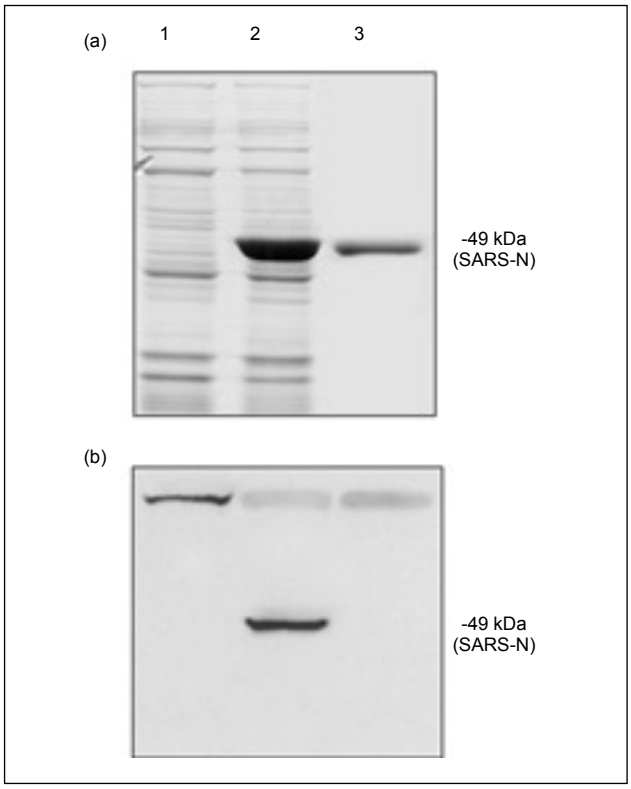

Fig 1. (a) Expression of SARS-CoV N protein in BL21-CodonPlus (DE3)-RIL
Coomassie blue staining: lane 1, total cell lysates before IPTG induction; lane 2, total cell lysates after 4 h IPTG induction at 30°C; lane 3, purified recombinant N protein
(b) Expression of SARS-CoV N protein in infected HEK-293 cells
Lane 1, uninfected cell lysates; lane 2, rAd-N infected cell lysates; and lane 3, rAd-LacZ infected cell lysates

of the immune system's weaponry against viral infection. The specific cellular response elicited by the rAd-N after immunisation was assessed by the secretion of IFN-γ by mouse splenocytes stimulated in vitro with recombinant SARS-CoV N protein for 40 h. The results clearly showed that rAd-N immunisation stimulated a substantial elevation of the IFN-γ-secreting T-cell response upon N protein stimulation compared with rAd-LacZ and PBS immunisation (Fig 3). This suggests that immunisation with rAd-N was able to trigger a SARS-CoV–specific T-cell response in the HLA-A2.1/Kb transgenic mice.

An effective vaccine must be able to raise a protective response from B and T cells after exposure to the viral agent. Different approaches have been used to produce a vaccine against SARS-CoV infection, including the use of inactivated SARS-CoV particles,[1] recombinant virus-like particles,[2] DNA,[3] recombinant proteins,[4] and recombinant viruses.[5]

In this study, a recombinant replication-defective adenovirus encoding the SARS-CoV nucleocapsid protein, rAd-N, was constructed (Fig 1) and its ability to induce the production of anti-SARS CoV N antibodies and cellular

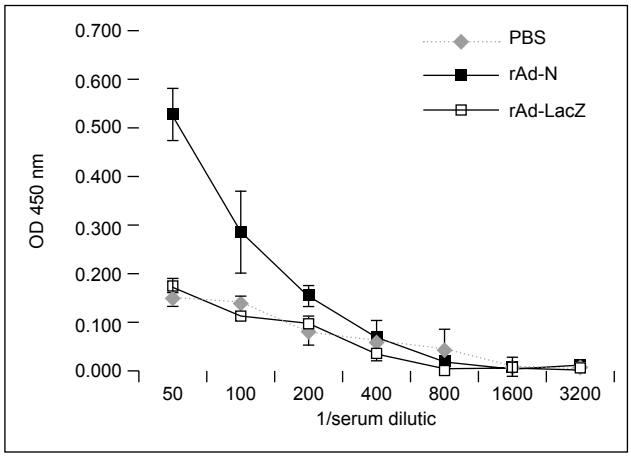

Fig 2. Antibody response against SARS-CoV N protein induced by rAd-N immunisation
Sera obtained from mice immunised either with 1×10^8 $TCID_{50}$ rAd-N, rAd-LacZ or PBS at 3 weeks after immunisation. The presence of anti-SARS-CoV N protein antibodies was detected by ELISA. Values represent the mean OD values measured from the sera of three individual mice

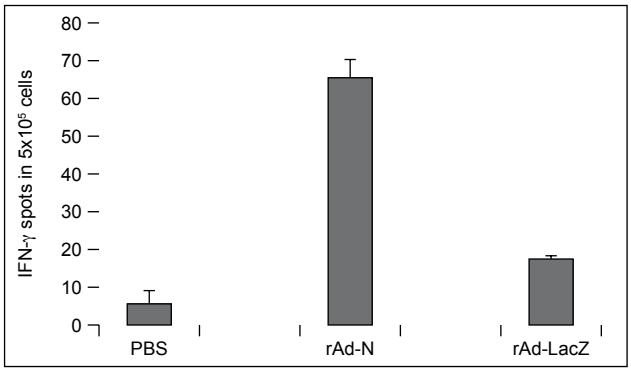

Fig 3. Cellular immune response induced by rAd-N immunisation
Splenocytes obtained from mice previously immunised either with 1×10^8 $TCID_{50}$ rAd-N, rAd-LacZ or PBS, were cultured in the presence of recombinant N protein. Secretion of IFN-γ was measured by ELISPOT. Results represent the mean±standard deviation (n=3, 5 mice per group)

immune responses was evaluated. Upon infection with the rAd, the infected HEK-293 cells expressed the SARS-CoV protein (Fig 1). The protein expressed within the infected cells was subjected to proteasome digestion and the resulting peptide was transported into the endoplasmic reticulum via the TAP protein, and eventually loaded onto the MHC class I molecule to elicit a cytotoxic T-cell response. Effective presentation of the viral derived peptides depends mainly on the high affinity binding of the MHC-I molecules towards the immunogenic peptides. Although previous studies have shown that the SARS-CoV protein expressed using adenoviral vectors is able to elicit an immune response against SARS protein, their assays were performed using normal murine models including C57, Balb/c and 129S6/SvEv. Since the peptide binding patterns of the MHC molecules from different animal species are so disparate, using normal murine models for such studies cannot reflect the T-cell response exhibited by humans. Our study used the HLA-A2.1/Kb transgenic mouse model which expresses human MHC-I molecules, and this model has been used for studying the immunogenicity of class I–restricted $CD8^+$ cytotoxic T lymphocyte (CTL) responses. In a previous study, the CTL response elicited by HLA-A2.1/Kb transgenic mice after infection with the influenza virus A/PR/8/34 was directed against the same dominant epitope recognised in humans expressing the HLA-A*0201 antigen. Subsequent studies using a panel of 38 different epitopes demonstrated that there is a good correlation between the CTL repertoire of these transgenic mice and HLA-A*0201–positive human individuals. Therefore, the model we used to investigate the immunogenicity of the rAd, rAd-N, is a more appropriate animal model for assessing the potency of a viral vaccine. The IFN-γ ELISPOT assay demonstrated that vaccination with rAd-N induces IFN-γ production from T cells upon SARS-CoV stimulation (Fig 3). Activation of T cells not only resulted in the elimination of infected cells, but the production of IFN-γ could also block or even lead to the elimination of virus from infected cells. Our ELISA data clearly show that the recombinant N protein can induce a strong humoral response against SARS-CoV N protein in vivo (Fig 2). Although the N protein is enclosed within the virus particle and expresses fewer neutralisation-mediated determinants, the antibody against the N protein can promote a T-cell response by forming an immune-complex through cross-presentation. Our results show that rAd-N can generate strong SARS-CoV–specific humoral and cellular immunity and may potentially be used as a SARS-CoV vaccine. Pre-existing immunity to wild-type human adenovirus in adult humans may affect the efficacy of Ad5-based vaccines against SARS-CoV. Nonetheless, modifying the adenovirus vector's surface chemically or with formulations able to mask antigenic determinants that can be recognised by neutralising antibodies may circumvent this problem. Also, the existence of approximately 50 identified adenovirus serotypes in humans and many other adenovirus of animal origin provides a great degree of genetic flexibility for designing sequential vaccine vectors. Overall this study provides valuable information aiding development of anti-SARS vaccines and other vaccine candidates for disease prevention in the future.

Acknowledgement

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References

1. Zhang CH, Lu JH, Wang YF, et al. Immune responses in Balb/c mice induced by a candidate SARS-CoV inactivated vaccine prepared from F69 strain. Vaccine 2005;23:3196-201.
2. Ho Y, Lin PH, Liu CY, Lee SP, Chao YC. Assembly of human severe acute respiratory syndrome coronavirus-like particles. Biochem Biophys Res Commun 2004;318:833-8.
3. Zhao P, Cao J, Zhao LJ, et al. Immune responses against SARS-coronavirus nucleocapsid protein induced by DNA vaccine. Virology

2005;331:128-35.
4. Bisht H, Roberts A, Vogel L, Subbarao K, Moss B. Neutralizing antibody and protective immunity to SARS coronavirus infection of mice induced by a soluble recombinant polypeptide containing an N-terminal segment of the spike glycoprotein. Virology 2005;334:160-5.
5. Kapadia SU, Rose JK, Lamirande E, Vogel L, Subbarao K, Roberts A. Long-term protection from SARS coronavirus infection conferred by a single immunization with an attenuated VSV-based vaccine. Virology 2005;340:174-82.

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Roles of spike protein in the pathogenesis of SARS coronavirus

Key Messages

1. Infection with SARS coronavirus (SARS-CoV) induces a cellular stress condition known as the unfolded protein response (UPR). UPR induction is mediated primarily by viral spike (S) protein. The modulation of UPR by S protein involves activation of PERK protein kinase. Other branches of the UPR pathways controlled by IRE1 and ATF6 proteins, respectively, are not involved.
2. The protease inhibitor Ben-HCl effectively suppresses SARS-CoV infection by blocking virus entry. Viral infectivity is associated with the cleavage of S protein by the cellular protease factor Xa.
3. Two new aspects of the interaction between SARS-CoV S protein and the cell have been defined. These have important implications in the pathogenesis of SARS, providing opportunities for developing vaccines and antivirals against SARS-CoV.
4. Counteracting the UPR and targeting the cleavage of S protein with small molecule pharmaceutical agents represent two new anti-SARS-CoV strategies.
5. The receptor-binding domain of S protein delivered via adeno-associated virus can efficiently induce mucosal immunity and provide long-term protection against SARS-CoV infection.

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Introduction

Severe acute respiratory syndrome (SARS) is a potentially fatal infectious disease caused by the SARS coronavirus (SARS-CoV). Like other coronaviruses, SARS-CoV is an enveloped and positive-stranded RNA virus that has a large genome of ~30 kb. It replicates in the cytoplasm and its life cycle is closely associated with the endoplasmic reticulum (ER). The viral activities have a profound impact on ER function. In particular, SARS-CoV hijacks the ER to process its structural and non-structural proteins.[1]

In eukaryotes, the ER is the processing factory for proteins destined for secretion or membrane insertion.[2] Accumulated nascent and unfolded SARS-CoV proteins in the ER lumen during replication can rapidly exceed its folding capacity, thereby perturbing its normal cellular function.

Perturbation of ER function causes stress. Stress of ER activates multiple cell signalling pathways to regulate gene expression at both transcriptional and translational levels. These pathways, collectively termed the unfolded protein response (UPR), adjust the biosynthetic burden and capacity of the ER to maintain homoeostasis. To date, three key proximal sensors of UPR, namely ATF6, IRE1 and PERK, have been identified.[2] Unfolded protein response can have both beneficial and detrimental effects during viral infection. To survive ER stress, viruses have developed different strategies to modulate UPR for their own benefits[3] but it is not known if, and in what ways, coronaviruses affect UPR in infected cells.

SARS-CoV S protein is a multifunctional protein that plays pivotal roles in the biology and pathogenesis of SARS-CoV. It has been shown that S protein mediates viral infection by binding to cellular receptor ACE2 and thus inducing membrane fusion. The other functional regions of S protein have not yet been defined. Specifically, it is not understood whether proteolytic cleavage of S affects viral infectivity. Our previous data suggest that the region close to the putative cleavage site might be influential in viral infection.[4]

The aim of this project was to shed light on the molecular and cellular basis of SARS-CoV pathogenesis.

Methods

This study was conducted from February 2005 to January 2007. The S gene and other viral genes of the SARS-CoV were subcloned and expressed in cultured mammalian cells. Pseudotyped SARS-CoV/HIV (pseudovirus) bearing S protein of SARS-CoV was also constructed. Properties of S protein were characterised in S-gene-transfected, SARS-CoV-infected and pseudovirus-infected cells using Western blotting, luciferase reporter assay, confocal immunofluorescence microscopy and immunoprecipitation. Additionally, recombinant S protein was also obtained and biochemically analysed in vitro.

Results

Infection with SARS-CoV induces ER stress

To investigate whether infection with SARS-CoV might have an impact on ER stress, we used commercial antibodies for GRP78/94 to determine whether their

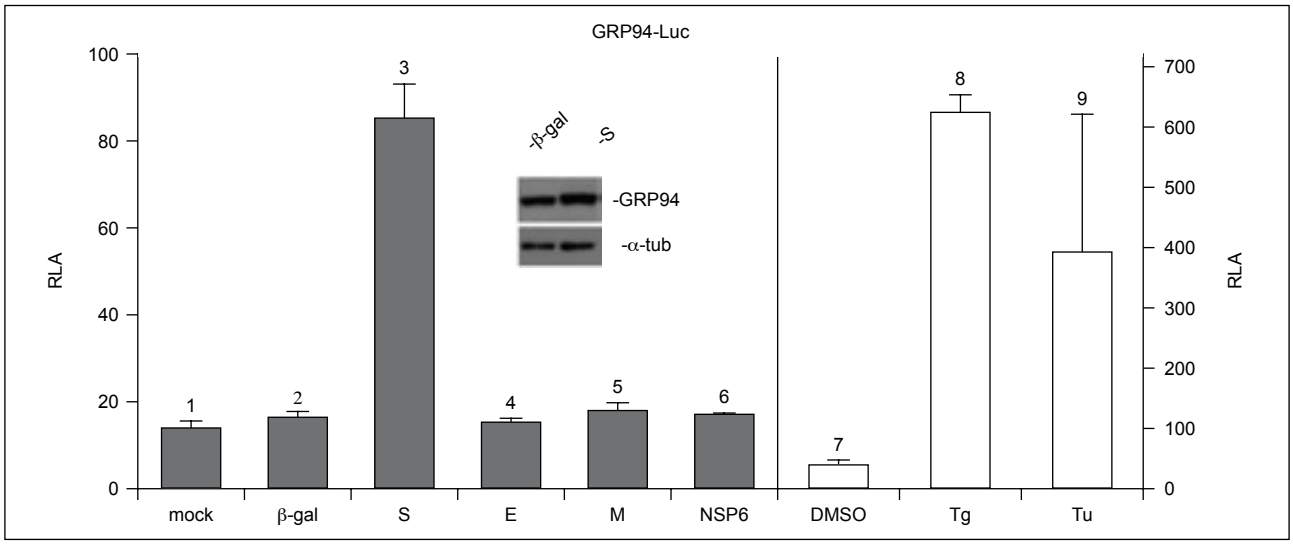

Fig 1. Influence of SARS-CoV proteins on UPR: SARS-CoV S protein activates GRP94 promoter (reproduced with permission from the American Society for Microbiology)

293FT cells were transfected with pGRP94-Luc plus an expression vector for the indicated protein. Control cells transfected with pGRP94-Luc alone were treated with DMSO, Tg (300 nM) or Tu (5 μg/ml) for 16 hrs. Cells were harvested 48 hrs post transfection for dual luciferase assay. Expression levels of GRP94 and α-tubulin (α-tub) in β-galactosidase (β-gal)- and S-expressing cells were verified by Western blotting (inset)

expression is induced in SARS-CoV-infected FRhK4 cells. We detected a 4.8-fold increase in the steady-state level of GRP94 in SARS-CoV-infected cells.

To further analyse the influence of SARS-CoV infection on transcriptional activation of GRP94 and GRP78 genes, we transfected luciferase reporter constructs driven by GRP94/78 promoters into Vero cells before infection with SARS-CoV. Our results indicated that infection with SARS-CoV induces ER stress through transcriptional activation of GRP78/94.

ER stress is induced by SARS-CoV S protein

To investigate whether different SARS-CoV membrane proteins might perturb the function of ER leading to UPR, we expressed SARS-CoV S, E, M and NSP6 proteins in 293FT cells. We observed that of the four, only S activated transcription from GRP94/78 promoters to ~5-fold (Fig 1, column 3 compared with columns 1 and 2). In the same experiment, treatment with thapsigargin (Tg) and tunicamycin (Tu), two well-known stimuli of ER stress,[2] led to 10~30-fold activation of luciferase expression (Fig 1, columns 8 and 9 compared with column 7). In contrast, none of the other three proteins significantly stimulated GRP94/78 promoters. The activation of GRP94 expression was also confirmed by Western blotting, which showed a 2.4-fold increase of GRP94 protein level in S-expressing cells as normalised to the level of α-tubulin (Fig 1, inset). Hence, activation of UPR by SARS-CoV is mediated at least partly through S protein.

Differential regulation of UPR pathways by SARS-CoV S protein

Stress of ER induces three major pathways of UPR signalling that are mediated through PERK, IRE1 and ATF6, respectively.[2] GRP94/78 promoters have been shown to be upregulated in response to PERK activation and eIF2α phosphorylation.[4] To investigate whether the activation of GRP94/78 promoters by S protein might be mediated through PERK and eIF2α, we employed PERK, eIF2α and their dominant negative (DN) or dominant active (DA) mutants. Interestingly, PERK DN and eIF2α DN effectively blocked basal and S protein-induced activation of GRP94/78 promoters (Fig 2, columns 3 and 4 compared with columns 1 and 2), whereas PERK wild-type and eIF2α DA stimulated these promoters (Fig 2, columns 5 and 6 compared with columns 1 and 2). Thus, PERK activity and eIF2α phosphorylation are required for the activation of ER stress by S protein.

Protease inhibitor Ben-HCl efficiently suppresses SARS-CoV infection

To determine if proteolytic cleavage of S protein affects viral infectivity, we screened protease inhibitors for suppressive effects on SARS-CoV infection. Among 13 inhibitors tested, only Ben-HCl had inhibitory activity. In addition, experiments with pseudovirus suggest that Ben-HCl inhibits viral entry into target cells.

Cleavage of SARS-CoV S protein by factor Xa and its inhibition by Ben-HCl

Since Ben-HCl is an inhibitor of a panel of proteases, we tested three proteases in this panel, including factor Xa, thrombin and trypsin, for their activities to cleave full-length recombinant S protein of SARS-CoV. Only factor Xa was able to effectively cleave SARS-CoV S protein into S1 and S2 subunits. This cleavage was effectively inhibited by 20 mM Ben-HCl. Similar results were obtained with S protein

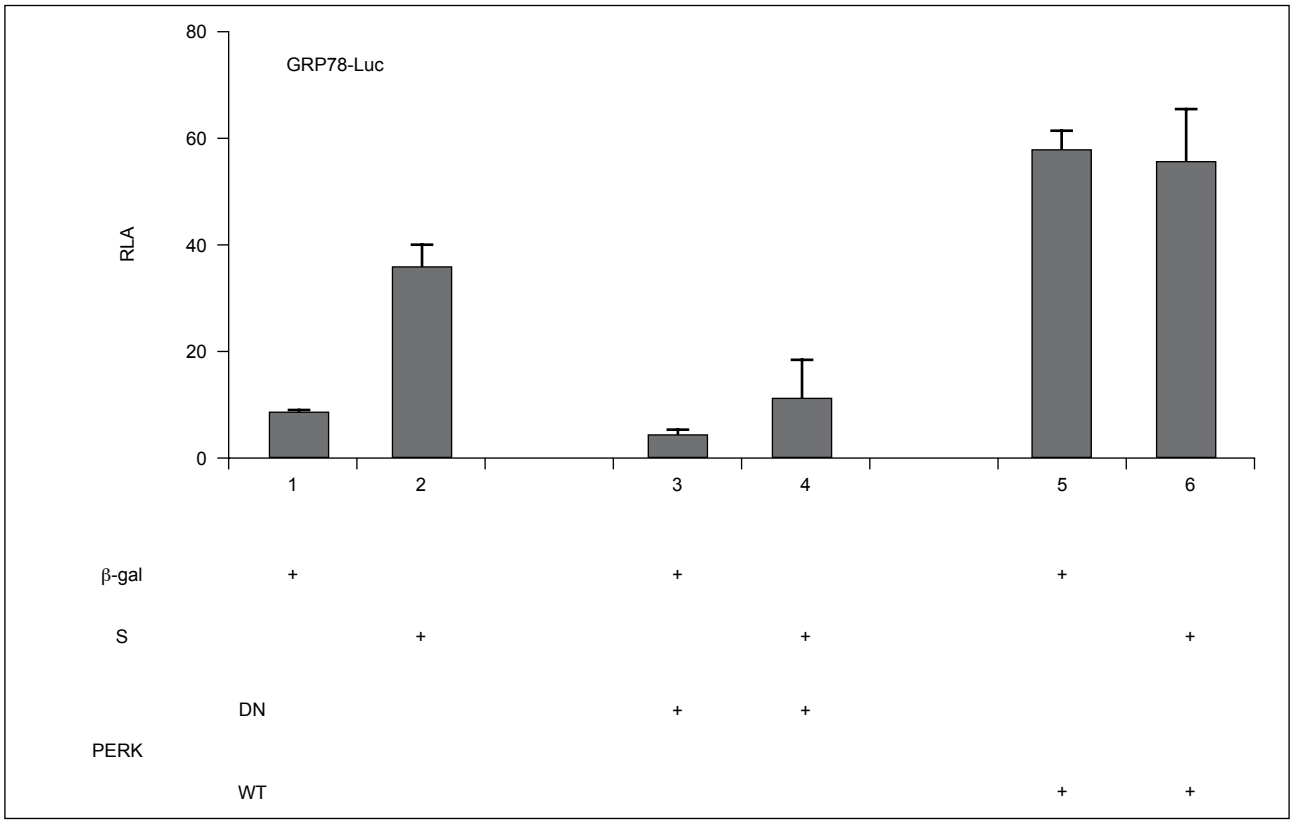

Fig 2. Activation of GRP78 by SARS-CoV S protein requires PERK (reproduced with permission from the American Society for Microbiology)

293FT cells were co-transfected with pGRP78-Luc and expression vectors for the indicated combinations of proteins. Cells were harvested for dual luciferase assay as in Figure 1

in pseudotyped SARS-CoV/HIV. Thus, both recombinant and pseudoviral S protein can be cleaved by factor Xa.

S protein was cleaved when the pseudovirus was incubated with the target cells

To determine if the infectivity of the SARS-CoV/HIV pseudovirus is indeed associated with the cleavage of the S protein by proteases on the target cell membrane, we tested the cleavage of S protein in the culture supernatant by Western blotting, and the infectivity of the pseudovirus in cell lysate using a luciferase assay. Our results indicated that the infectivity of the pseudovirus increased with time and correlated with the size of the cleavage products. The expression of factor Xa in 293T/ACE2 cells was further confirmed by RT-PCR and Western blotting.

Intranasal vaccination of recombinant AAV encoding RBD of S potently induces mucosal immune responses and provides long-term protection against SARS-CoV infection

Systemic, mucosal, and cellular immune responses and long-term protective immunity induced by RBD-AAV were characterised in a BALB/c mouse model, with comparison of the intramuscular and intranasal routes of administration. Our findings suggest that RBD-AAV can be further developed into a candidate vaccine against SARS. Intranasal vaccination may be the preferred route of administration due to its ability to induce SARS-CoV-specific systemic and mucosal immune responses and its better safety profile.

Discussion

Our demonstration of the modulation of ER stress and UPR by SARS-CoV S protein suggests a new role for the S protein after viral entry. This modulation of UPR probably represents a viral strategy to combat the cellular response and to facilitate viral replication. On the other hand, induction of ER stress by S protein has a significant impact on cell homoeostasis and may contribute to viral pathogenesis. For example, UPR is activated in response to the release of ER calcium and it will be of interest to see whether SARS-CoV might induce sufficient calcium release from ER and cause diarrhoea by acting as an NSP4-like viral enterotoxin.[5]

Modulation of ER stress and UPR by the SARS-CoV reveals a novel opportunity for pharmaceutical intervention in SARS. Due to the importance of ER stress in various human diseases including viral infection, small molecules that specifically counteract ER stress have been under intense investigations.[2] In this regard, one selective inhibitor of eIF2α dephosphorylation has recently been found to be effective for the inhibition of herpes simplex virus replication.[6] Additionally, drugs that modulate ER stress

have also been shown to inhibit the production of infectious CMV virions.[7] As we are yet to identify effective antivirals for the treatment of SARS,[1] further investigation of the use of various ER stress-modulating pharmaceutical agents for anti-SARS-CoV therapy is warranted.

Our findings that SARS-CoV S protein can be cleaved by factor Xa into S1 and S2 subunits both in vitro and in mammalian cells suggests a plausible mechanism by which SARS-CoV cleaves S protein to facilitate viral infection. As inhibition of this cleavage, using agents such as Ben-HCl, can effectively block viral entry, our work has provided a new target for the development of anti-SARS agents. Additionally, the region surrounding the cleavage site should also be included in candidate vaccines against SARS-CoV.

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Results of this study were published in the following four papers: (1) Chan CP, Siu KL, Chin KT, Yuen KY, Zheng B, Jin DY. Modulation of the unfolded protein response by the severe acute respiratory syndrome coronavirus spike protein. J Virol 2006;80:9279-87. (2) Du L, Kao RY, Zhou Y, et al. Cleavage of spike protein of SARS coronavirus by protease factor Xa is associated with viral infectivity. Biochem Biophys Res Commun 2007;359:174-9. (3) Du L, Zhao G, Lin Y, et al. Intranasal vaccination of recombinant adeno-associated virus encoding receptor-binding domain of severe acute respiratory syndrome coronavirus (SARS-CoV) spike protein induces strong mucosal immune responses and provides long-term protection against SARS-CoV infection. J Immunol 2008;180:948-56. (4) Du L, Zhao G, Lin Y, et al. Priming with rAAV encoding RBD of SARS-CoV S protein and boosting with RBD-specific peptides for T cell epitopes elevated humoral and cellular immune responses against SARS-CoV infection. Vaccine 2008;26:1644-51.

References

1. Weiss SR, Navas-Martin S. Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev 2005;69:635-64.
2. Schröder M, Kaufman RJ. The mammalian unfolded protein response. Annu Rev Biochem 2005;74:739-89.
3. He B. Viruses, endoplasmic reticulum stress, and interferon responses. Cell Death Differ 2006;13:393-403.
4. Zheng BJ, Guan Y, Hez ML, et al. Synthetic peptides outside the spike protein heptad repeat regions as potent inhibitors of SARS-associated coronavirus. Antivir Ther 2005;10:393-403.
5. Ball JM, Tian P, Zeng CQ, Morris AP, Estes MK. Age-dependent diarrhea induced by a rotaviral non-structural glycoprotein. Science 1996;272:101-4.
6. Boyce M, Bryant KF, Jousse C, et al. A selective inhibitor of eIF2alpha dephosphorylation protects cells from ER stress. Science 2005;307:935-9.
7. Isler JA, Maguire TG, Alwine JC. Production of infectious human cytomegalovirus virions is inhibited by drugs that disrupt calcium homoeostasis in the endoplasmic reticulum. J Virol 2005;79:15388-97.

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An oral mucosal DNA vaccine for SARS coronavirus infections

Key Messages

1. When different forms of SARS coronavirus (SARS-CoV) spike protein-based vaccines for generation of a neutralising antibody response to SARS-CoV were injected into a mouse model, all the mice immunised with intramuscular tPA-optimise800 DNA vaccine boosted with intraperitoneal recombinant spike polypeptide generated by *Escherichia coli* and intramuscular CTLA4HingeSARS800 DNA vaccine boosted with intraperitoneal S-peptide had neutralising antibody titres of ≥1:1280.
2. This observation may have major practical value for field studies, such as the immunisation of civet cats, as the cost of recombinant proteins produced by *E coli* is much lower than those produced by eukaryotic systems.
3. This study indicates that the type of vaccine used for priming is crucial for determining the type of immune response developed. Subsequent doses will boost the immune response generated by the first dose of vaccine.

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Introduction

The 2003 severe acute respiratory syndrome (SARS) outbreak was the first epidemic caused by a coronavirus and resulted in a fatality rate of approximately 10%.

Coronavirus spike proteins have been shown to be highly immunogenic, and able to produce neutralising antibodies effective for prevention of infections caused by the corresponding coronaviruses when introduced into animals. There are no data on less expensive modalities of immunisation, such as DNA vaccination followed by booster doses of recombinant vaccine produced by *Escherichia coli* or oral mucosal DNA vaccines.

Methods

This study was conducted from September 2004 to August 2006.

Study design

To compare the neutralising antibody response to SARS-CoV generated by different forms of SARS-CoV spike protein-based vaccines comparison groups of mice were immunised with the following vaccines: recombinant spike polypeptide vaccine produced by *E coli*, two different types of intramuscular spike polypeptide DNA vaccine with and without boosters of recombinant spike polypeptide vaccine produced by *E coli* and two different types of oral mucosal spike polypeptide DNA vaccine with and without boosters of recombinant spike polypeptide vaccine produced by *E coli*.

Animals and immunisation schedule, ELISA, and neutralising antibody assay

Details of the experimental protocol have been reported.[1]

Results

Among all groups of mice, sera of all the mice immunised with i.m. tPA-S-DNA boosted with i.p. S-peptide and i.m. CTLA4-S-DNA boosted with i.p. S-peptide showed the highest neutralising antibody titres of ≥l:1280. Details of the results have been reported.[1]

Discussion

Of all groups, the mice primed with SARS-CoV human-codon-usage-optimised spike polypeptide DNA vaccines and boosted with S-peptide produced by *E coli* generated the highest neutralising antibody titres against SARS-CoV. It has been observed, and was confirmed by the present study, that S-peptide produced by *E coli* does not induce neutralising antibodies to SARS-CoV infection. This is probably because when S-peptide produced by *E coli* was used, the three dimensional folding and/or the glycosylation of the S-peptide was not optimal for the generation of neutralising antibodies. In this study, we documented that, although recombinant S-peptide produced by *E coli* itself was not able to generate neutralising antibodies against SARS-CoV infection, mice primed with spike polypeptide DNA vaccine and boosted with S-peptide from *E coli* were able to generate high titres of neutralising antibody against SARS-CoV. This indicates that the type of vaccine used for priming is crucial for determining the

type of immune response that develops. Subsequent doses will boost the immune response generated by the first dose of vaccine.

This observation may have major practical value in areas such as the immunisation of civet cats. Production of recombinant proteins from *E coli* is far less expensive than production of recombinant proteins using eukaryotic systems, such as transfection of cell lines, or DNA vaccines. Although it has been shown that DNA vaccines are able to successfully generate both humoral and cellular immunity to various pathogens in mice, one of the major limitations for their clinical use is their ineffectiveness when used in humans, unless a large amount of DNA is used for immunisation. It is difficult to scale up the levels of production of eukaryote-generated recombinant proteins to industrial levels. Therefore, the large amount of S-peptide that is produced by *E coli* in a relatively inexpensive way could be used as booster doses instead of being injected alone as vaccine. This principle can also be examined in vaccination for other pathogens, where 'more effective' modalities of vaccination, such as DNA vaccines, can be used for priming, and the 'less expensive' recombinant protein produced by *E coli*, instead of eukaryotic systems, can be used as booster doses.

Acknowledgement

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Reference

1. Woo PC, Lau SK, Tsoi HW, et al. SARS coronavirus spike polypeptide DNA vaccine priming with recombinant spike polypeptide from *Escherichia coli* as booster induces high titer of neutralizing antibody against SARS coronavirus. Vaccine 2005;23:4959-68.

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Association of cytokine and chemokine gene polymorphisms with severe acute respiratory syndrome

Key Messages

1. The *IFN-γ* +874A allele and *RANTES* -28 G allele are risk factors for SARS susceptibility.
2. The *RANTES* -28 G allele plays a role in the pathogenesis of SARS.
3. The polymorphisms of *IL-10, TNF-α, IL-12, IP-10, Mig* and *MCP-1* are not associated with SARS susceptibility.

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Introduction

Severe acute respiratory syndrome (SARS) is an infectious disease caused by the SARS coronavirus,[1] but the pathogenesis is still far from clear.[2] We have demonstrated that genetic haplotypes associated with low-serum mannose-binding lectin (MBL) are associated with SARS,[3] and our findings have been replicated recently.[4] Hong Kong Chinese who are homozygotes for *CLEC4M* tandem repeats have recently been reported to be less susceptible to SARS. Other susceptibility genes, such as OAS-1 and MxA have also been identified.

Cytokines are known to take an important role in antiviral action. Interferon (IFN)-γ from T and natural killer (NK) cells is important for driving the T helper cell type 1 (Th1) responses. It also activates monocytes and macrophages, which in turn take part in antiviral responses by producing free radicals and pro-inflammatory cytokines like tumour necrosis factor (TNF)-α. On the other hand, interleukin (IL)-10 counteracts the inflammatory response by inhibiting TNF-α production and neutrophil activation. Interleukin-12 is important for the development of the T helper type 1 (Th1) response in the initial phase of bacterial, parasitic, and viral infections. In many viral infections, IL-12 promotes viral clearance and host recovery from infection.

Chemokines play an important role in cell trafficking during immune responses. Acute respiratory viruses commonly induce inflammatory chemokines in local tissue. In a previous study we confirmed that the SARS coronavirus induces upregulation of a number of inflammatory chemokines, ie Regulated upon Activation Normal T cell-Expressed and Secreted (RANTES), interferon-gamma inducible protein 10 (IP-10) and Monocyte Chemoattractant Protein-1 (MCP-1). The upregulation of these chemokines, including the monokine induced by interferon gene (Mig) may recruit inflammatory cells and leukocytes into the tissue.

In this study, we hypothesised that polymorphisms of the cytokine genes, *IFN-γ, IL-10, TNF-α*, *IL-12,* and the chemokine genes, *RANTES, IP-10, Mig* and *MCP-1*, might be associated with SARS. These genes were chosen for their key roles in antiviral action and inflammation regulation and their polymorphisms based on their potential regulation of gene expression.

Methods

This study was conducted from September 2005 to August 2006.

Patient populations

The study was approved by the Clinical Research Ethics Committee of the Institutional Review Board of the University of Hong Kong/Hospital Authority Hong Kong West Cluster. This study included 495 Hong Kong Chinese patients with SARS (211 males and 284 females, mean±SD age was 40.74±15.73 years) and 578 ethnically matched healthy controls from the Red Cross (343 males and 235 females, mean±SD age was 30.05±9.49 years). At least 95% of the patients were documented as having SARS-CoV antibody seroconversion and/or detectable SARS-CoV RNA in respiratory secretions by RT-PCR. The death group consisted of 57 patients who died from SARS and their mean±SD age was

Table 1. Genotype frequencies and allele frequencies of cytokines in SARS patients and controls

Genotype	SNP	No. (%)		OR (95% CI)*	P value*
		SARS (n=476)	Control (n=449)		
IFN-γ +874	A/A	332 (69.8)	203 (45.2)	5.19 (2.78 - 9.68)	<0.001
	A/T	127 (26.7)	189 (42.1)	2.57 (1.35 - 4.88)	
	T/T	17 (3.6)	57 (12.7)	Reference	
IL-10 -1082	A/A	439 (92.2)	411 (91.5)		NS†
	A/G	35 (7.4)	38 (8.5)		
	G/G	2 (0.4)	0 (0)		
IL-10 -592	A/A	244 (51.3)	209 (46.6)		NS
	A/C	188 (39.5)	214 (47.7)		
	C/C	44 (9.2)	26 (5.8)		
TNF-α -308	GG	403 (84.7)	377 (83.9)		NS
	GA	70 (14.7)	70 (15.6)		
	AA	3 (0.6)	2 (0.5)		
		SARS (n=470)	Control (n=558)		
IL-12B promoter 318/322	318/318	122 (26.0)	145 (26.4)		NS
	318/322	252 (53.6)	238 (43.4)		
	322/322	96 (20.4)	166 (30.2)		
IL-12B intron 2 218/221	218/218	153 (32.6)	160 (29.3)		NS
	218/221	234 (49.8)	251 (45.9)		
	221/221	83 (17.7)	135 (24.7)		
IL-12B intron 4 268/272	268/268	70 (14.9)	133 (23.8)		NS
	268/272	222 (47.2)	237 (42.5)		
	272/272	147 (31.3)	150 (26.9)		
	Others	31 (6.6)	38 (6.81)		
IL-12B 3'UTR	A/A	157 (33.4)	155 (27.9)		NS
	A/C	235 (50.0)	294 (52.9)		
	C/C	78 (16.6)	107 (19.2)		
Allele					
IFN-γ +874	A	791 (83.1)	595 (66.3)	2.23 (1.75 - 2.83)	<0.001
	T	161 (16.9)	303 (33.7)		
IL-10 -1082	A	913 (95.9)	860 (95.8)		NS
	G	39 (4.1)	38 (4.2)		
IL-10 -592	A	676 (71.0)	632 (70.4)		NS
	C	276 (29.0)	266 (29.6)		
TNF-α -308	G	876 (92.0)	824 (91.8)		NS
	A	76 (8.0)	74 (8.2)		
IL-12B promoter 318/322	318	496 (52.8)	528 (48.1)		NS
	322	444 (47.2)	570 (51.9)		
IL-12B intron 2 218/221	218	540 (57.5)	571 (52.3)		NS
	221	400 (42.6)	521 (47.7)		
IL-12B intron 4 268/272	268	376 (40.0)	517 (46.4)		NS
	272	533 (56.7)	560 (50.3)		
	Others	31 (3.3)	37 (3.30)		
IL-12B 3'UTR	A	549 (58.4)	604 (54.3)		NS
	C	391 (41.6)	508 (45.7)		

* P value and OR (95% CI) were calculated with the use of logistic regression models, adjusted for sex and age. After correction by Bonferroni method, the significant P value should be less than 0.003

† NS denotes not significant

56.2±15.3 years, with 33 males and 24 females.

Genotyping

IFN-γ +874A/T, *IL-10* -1082G/A, *IL-10* -592A/C, *IL-12B* promoter 318/322, *IL-12B* intron 2 218/221, *IL-12B* intron 4 268/272, *IL-12B* 3'UTR A/C and *TNF-α* -308 G/A were genotyped using the TaqMan system (Applied Biosystems, CA, USA). *RANTES* -28C/G and *MCP-1* -2518A/G were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). *RANTES* -403A/G, *RANTES* In1.1T/C, *IP-10* nt1811A/G, *IP-10* nt2867C/A and *Mig* nt367A/G were genotyped using the MassARRAY system (Sequenom, CA, US).

Statistical analysis

A two-step analysis was used to determine the association of polymorphisms with SARS. The genotype frequencies and allele frequencies of all the genes were compared between SARS patients and controls using a 3x2 Chi squared test and a 2x2 Chi squared test respectively. Logistic regression was then used to calculate the odds ratios (OR) [95% confidence interval (CI)] and corresponding P values of different genotype frequencies among SARS patients and controls by adjusting for age and sex as co-variables. Association with SARS infection outcomes (death vs survival) was then tested by comparing the genotype frequencies and allele frequencies of all the genes between the death group and the survival group of SARS patients using a 3x2 Chi squared test and a 2x2 Chi squared test respectively. The genotype frequencies of all the single nucleotide polymorphisms (SNPs) were tested for Hardy-Weinberg equilibrium (HWE) separately in SARS patients and controls using the Chi squared test. The significant P value for multiple testing was adjusted with Bonferroni's correction and all statistical analyses were performed using SAS, version 8.02 and SAS/Genetics (SAS Institute, NC, US).

Table 2. Genotype frequencies and allele frequencies of chemokines in SARS patients and controls

Genotype	SNP	No. (%)		OR (95% CI)*	P value*
		SARS (n=495)	Control (n=578)		
RANTES -403	AA	54 (10.9)	56 (9.7)		NS†
	AG	223 (45.0)	262 (45.3)		
	GG	218 (44.0)	260 (45.0)		
RANTES -28	CC	316 (63.8)	491 (84.9)	Reference	<0.0001
	CG	154 (31.1)	73 (12.6)	3.28 (2.32 - 4.64)	
	GG	25 (5.0)	14 (2.4)	3.06 (1.47 - 6.39)	
RANTES In1.1	CC	54 (10.9)	54 (9.3)		NS
	CT	217 (43.8)	257 (44.5)		
	TT	224 (45.3)	267 (46.2)		
IP-10 nt1811	AA	1 (0.2)	0 (0)		NS
	AG	38 (7.7)	29 (5.0)		
	GG	456 (92.1)	549 (95.0)		
IP-10 nt2867	AA	1 (0.2)	0 (0)		NS
	AC	38 (7.7)	31 (5.4)		
	CC	456 (92.1)	547 (94.6)		
Mig nt367	AA	1 (0.2)	0 (0%)		NS
	AG	38 (7.7)	34 (5.9)		
	GG	456 (92.1)	544 (94.1)		
		SARS (n=478)	Control (n=421)		
MCP-1 -2518	AA	115 (24.1)	113 (26.8)		NS
	AG	225 (47.1)	213 (50.6)		
	GG	138 (28.9)	95 (22.6)		
Allele					
RANTES -403	A	331 (33.4%)	374 (32.4%)		NS
	G	659 (66.6%)	782 (67.7%)		
RANTES -28	C	786 (79.4%)	1055 (91.3%)	2.80 (2.11 - 3.71)	<0.0001
	G	204 (20.6%)	101 (8.7%)		
RANTES In1.1	C	325 (32.9%)	365 (31.6%)		NS
	T	665 (67.2%)	791 (68.4%)		
IP-10 nt1811	A	40 (4.0%)	29 (2.5%)		NS
	G	950 (96.0%)	1127 (97.5%)		
IP-10 nt2867	A	40 (4.0%)	31 (2.7%)		NS
	C	950 (96.0%)	1125 (97.3%)		
Mig nt367	A	40 (4.1%)	34 (2.9%)		NS
	G	938 (95.9%)	1122 (97.1%)		
MCP-1 -2518	A	455 (47.6)	439 (52.1)		NS
	G	501 (52.4)	403 (47.9)		

* P value and OR (95% CI) were calculated with the use of logistic regression models, adjusted for sex and age. After correction by Bonferroni method, the significant P value should be less than 0.003

† NS denotes not significant

Results

Our case-control study for cytokines genotyped the SNPs *IFN-γ* +874A/T, *IL-10* -1082G/A, *IL-10* -592A/C, *TNF-α* -308G/A, *IL-12B* promoter 318/322, *IL-12B* intron 2 218/221, *IL-12B* intron 4 268/272 and *IL-12B* 3'UTR A/C in Chinese patients with SARS and in healthy controls. All SNPs were in HWE (P>0.05) in SARS patients and controls using the Chi squared test and the genotype distributions and allele frequencies of these SNPs are shown in Table 1. The *IFN-γ* +874A allele was overrepresented in the SARS patients (83.1%) when compared with the controls (66.3%) [P<0.001]. It was also significantly associated with susceptibility to SARS in a dose-dependent manner (P<0.001), ie individuals with *IFN-γ* +874 AA and AT genotype had OR of 5.19 (95% CI, 2.78-9.68) and 2.57 (95% CI, 1.35-4.88) of developing SARS respectively. The SNPs of *IL-10, TNF-α*, and *IL-12* SNPs were chosen for their potential regulation of protein expression levels. Nonetheless we found no significant association between these SNPs and SARS (Table 1). We also compared the genotype and allele frequencies of all the polymorphisms of the SARS patient death and survival groups but no significant associations were established.

For the study on chemokines, *RANTES* -28C/G, *RANTES* -403A/G, *RANTES* In1.1T/C, *IP-10* nt1811A/G, *IP-10* nt2867C/A, *Mig* nt367A/G, and *MCP-1* -2518 A/G were genotyped. Their genotype and allele frequencies are shown in Table 2. The *RANTES* -28 CG and GG genotypes were significantly associated with SARS susceptibility with OR of 3.28 (95% CI, 2.32-4.64) and 3.06 (95% CI, 1.47-6.39) respectively (P<0.0001) [Table 2]. The *RANTES* -28 G allele was also significantly increased in SARS patients (P<0.0001, OR=2.80, 95% CI, 2.11-3.71) [Table 2]. Our data did not show any significant association with the SNPs of *IP-10*, *Mig* and *MCP-1* (Table 2). All genotype frequencies of the chemokine polymorphisms in SARS patients and controls were in HWE except for the *RANTES* -28C/G frequency in the controls. To confirm that there was no genotyping error that may have contributed to the HWE observation, direct DNA sequencing was performed on 20 to 30 samples for each SNP. No ambiguous results were obtained. We further compared the genotype and allele frequencies of the *RANTES* -28C/G between the SARS death and survival groups. The *RANTES* -28 G allele was associated with death from SARS in a gene-dosage dependent manner (P=0.014), with -28 CG and GG individuals having a 2.12-fold (95% CI, 1.11-4.06) and 4.01-

Table 3. Genotype and allele frequencies of *RANTES* -28C/G among death and survival groups in SARS patients

RANTES -28C/G	No. (%)		OR (95% CI)*	P value*
	Death (n=57)	Survival (n=438)		
Genotype				0.014
CC	26 (45.6)	290 (66.2)	Reference	
CG	25 (43.9)	129 (29.5)	2.12 (1.11 - 4.06)	
GG	6 (10.5)	19 (4.3)	4.01 (1.30 - 12.4)	
Allele				0.002
C	77 (67.5)	709 (80.9)		
G	37 (32.5)	167 (19.1)	2.10 (1.30 - 3.39)	

* P value and OR (95% CI) were calculated with the use of logistic regression models, adjusted for sex and age

fold (95% CI, 1.30-12.4) increased risk of death from SARS respectively (Table 3). No association with death from SARS was detected for the other chemokine genes studied.

Discussion

It has been reported previously that the *IFN-γ* +874A allele is associated with infectious diseases, revealing its potential role in host defences against microbial infections. The mechanism by which the *IFN-γ* +874A/T allele influences susceptibility to SARS may depend on its role in the regulation of IFN-γ production. The T allele of *IFN-γ* +874A/T provides a binding site for the transcription factor nuclear factor-kB (NF-kB), which is able to regulate IFN-γ expression. It is possible that low IFN-γ production may impair the anti-viral response to SARS-CoV, rendering these individuals more susceptible to infection with this virus. Our observation that the *IFN-γ* +874A allele was significantly associated with SARS-CoV infection suggests a genetic risk factor for SARS. The role of IFN-γ in the antiviral response to SARS-CoV has also been supported by recent studies showing that IFN-γ can inhibit the replication of SARS-CoV in combination with IFN-β in vitro.

RANTES is responsible for the recruitment of eosinophils, lymphocytes, monocytes and basophils at the site of inflammation and is involved in many viral infections. We found that the -28 G allele of *RANTES* was associated with susceptibility to and death from SARS. *RANTES* -28C/G is located at the NF-κB binding site, suggesting that this SNP may be involved in the regulation of RANTES expression. Further in vitro studies have shown that the *RANTES* -28 G allele enhances NF-κB binding that leads to elevation of promoter activity and increases RANTES expression in CD8+ T cells, CD4+ T cells and monocytes/macrophages. Together with our observation that the -28 G allele is associated with SARS, we conclude a high level of RANTES may predispose individuals to developing SARS. Too high a level of RANTES may intensify lung inflammation and lead to lymphopaenia, increasing the chance of secondary infection and hence the death rate among SARS patients. Therefore, we speculate that the *RANTES* -28 G allele that associates with higher levels of RANTES may enhance inflammation and lead to severe clinical outcomes in SARS. Indeed, the *RANTES* -28 G allele did show a strong association with death in Hong Kong Chinese patients with SARS (Table 3).

We did not find a significant association between the SNPs of *IL-10, TNF-α, IL-12, IP-10, Mig,* and *MCP-1* and SARS. Nevertheless, we cannot entirely exclude the roles of these cytokines and chemokines in susceptibility to SARS, because other SNPs in these genes may also be involved in gene expression regulation. Further studies on other SNPs able to alter gene expression levels are required to ascertain the relationship of these SNPs with SARS.

Conclusions

We demonstrated that the *IFN-γ* +874A allele is significantly associated with SARS susceptibility in a dose-dependent manner.[5] Due to its role in regulating IFN-γ expression, this allele may be involved in the pathogenesis of SARS by altering IFN-γ production. In addition, we demonstrated that the *RANTES* -28 G allele, which correlates with high RANTES production, was associated with SARS susceptibility.[6] It was also associated with adverse SARS outcomes, suggesting that a high RANTES level may play a role in the pathogenesis of SARS.[6]

Acknowledgements

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References

1. Peiris JS, Lai ST, Poon LL, et al. Coronavirus as a possible cause of severe acute respiratory syndrome. Lancet 2003;361:1319-25.
2. Lau YL, Peiris JS. Pathogenesis of severe acute respiratory syndrome. Curr Opin Immunol 2005;17:404-10.
3. Ip WK, Chan KH, Law HK, et al. Mannose-binding lectin in severe acute respiratory syndrome coronavirus infection. J Infect Dis 2005;191:1697-704.
4. Zhang H, Zhou G, Zhi L, et al. Association between mannose-binding lectin gene polymorphisms and susceptibility to severe acute respiratory syndrome coronavirus infection. J Infect Dis 2005;192:1355-61.
5. Chong WP, Ip WK, Tso GH, et al. The interferon gamma gene polymorphism +874 A/T is associated with severe acute respiratory syndrome. BMC Infect Dis 2006;6:82.
6. Ng MW, Zhou G, Chong WP, et al. The association of *RANTES* polymorphism with severe acute respiratory syndrome in Hong Kong and Beijing Chinese. BMC Infect Dis 2007;7:50.

AUTHOR INDEX

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