Original Article

T Lymphocyte Activation Profiles in Peripheral Blood of Long- Versus Short-Term Residents of Kuwait: Comparison with Asthmatics

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Abstract

Introduction: During the Arabian Gulf Wars of 1991 and 2003, the resident population of Kuwait sustained heavy exposure to environmental toxicants introduced by military activities. No comprehensive studies have been conducted to assess how exposure to the wartime and postwar environment may have altered the fundamental patterns of immune reactivity among Kuwaitis in ways that affect pathogenesis of disease. This present study addresses this issue by characterising immunological features of asthma and allergies in a Kuwaiti population that is unique and possibly correlates with toxicant exposures. Materials and Methods: Twenty-five long-term residents of Kuwait afflicted with bronchial asthma concurrent with rhinitis; and 2 healthy control groups: 18 long-term residents and 10 newcomers to Kuwait were evaluated by 2- and 3-colour flow cytometry for peripheral blood T cell subpopulation frequencies. Results: Relative to healthy, long-term residents, significantly elevated frequencies of all activated cell phenotypes were observed in the blood of the asthmatic group (P <0.05 to P <0.001), except for CD8+HLA-DR+ cells and a presumed T-regulatory (Treg) subpopulation: CD4+CD25^{high}. The asthmatic group was also observed to have larger populations of CD3+ (pan-T cells), CD4+ (T helper cells) and CD8+ (cytotoxic T cells), CD3+CD56 (NKT-like cells) and CD56+CD16+ (NK cells) compared to healthy long-term residents. Compared to healthy recent immigrants, the blood of long-term residents contained elevated levels of CD3+CD56+ (NK-like), CD4+CD45RA+/CD45RO+ (Naive-to-Memory Transitional), but lower CD4+CD25+^{high} (Treg) (P <0.05). Conclusions: Elevated representation of natural killer (NKT)-like and memory phenotypes may predispose long-term residents towards enhanced susceptibility for airway disease; while at the same time, reducing representation of Treg cells which are protective against airway disease, and this may increase vulnerability to these syndromes among the residents of Kuwait. These results may provide insight into the features of immunopathogenesis of asthma and allergies in Kuwait that arise as a result of the special environment of the country.

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Key words: Asthma, Immune reactivity, Kuwait, Rhinitis, T cells

Introduction

The Gulf Wars of 1991 and 2003 exposed the residents of Kuwait and several neighbouring countries to high levels toxicants with the capacity to cause long-term deleterious alterations in the immune reactivity.[1-7] The impact of these influences on the epidemiology and pathogenesis of chronic illness in affected populations is poorly characterised and remains largely speculative at the time of this writing.

This study examines the profiles of activated T cell phenotypes in the peripheral blood of healthy Kuwaiti residents in the country since the first Gulf War in 1991 in comparison with a group of healthy persons who recently arrived in the country, with another group of Kuwaiti afflicted simultaneously with asthma and rhinitis. This study represents a preliminary effort to establish trends of T cell activation among Kuwaitis suffering from respiratory disorders that may provide clues as to how the special environment of Kuwait affects the immune reactivity so as to contribute to the disease. A major focus of these investigations is syndromes arising from immune dysregulation in which pathogenic T lymphocytes play a major role.

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Disorders mediated by pathogenic T cells, which include asthma and allergic disease, are characterised by alterations in profiles of peripheral blood lymphocytes reflecting abnormal activity that ultimately results in symptoms of each disease. In asthma and other allergic diseases, a T lymphocyte subset of CD4 Th2 cells, induces eosinophilia in the lungs and respiratory mucosa, resulting in the aforementioned symptoms.[8-10] The central role of T lymphocytes and their products in the pathogenesis of a variety of diseases is well accepted. These cells are broadly characterised into 3 mechanistic subsets: T helper lymphocytes (THL), which express the CD4+ on their cell surface and affect cellular processes by production of cytokines and other soluble factors; and cytotoxic/suppressor T lymphocytes (CTL) which express the CD8 cell surface antigen as an identifying marker and regulate activity of other cells by direct contact. A regulatory species of CD4 T-helper cell (Treg) has also been identified and has become the focus of increasing interest. These cells, which express both CD4 and the activation marker CD25+ (the IL-2 receptor) on their cell surface, are able to suppress pathogenic T cell activity by expression of immunosuppressive soluble factors, such as the cytokine IL-10 and in doing so, serve as a major mechanism for prevention of autoimmunity and allergic disease.[11] In asthma, CD4+Th2 lymphocytes are known to produce a specific group of cytokines (IL-3, IL-5, and GM-CSF) that increase the recruitment, proliferation, differentiation, activation, and life span of eosinophils.[12,13] Such enhanced local recruitment, life span and activation of eosinophils by cytokines promotes tissue injury and fibrosis via an increased production of a variety of toxic factors including oxygen radicals, major basic proteins and neurotoxins, as well as regulatory cytokines such as transforming growth factor (TGF) beta in a diverse range of inflammatory pathologies.[14-18]

Other subpopulations of helper T cells which have also been implicated as agents of chronic inflammatory disease are Th1 cells, which typically respond to antigenic stimulation by expression of interleukin-2 (IL-2) and interferon-gamma (IFN-γ). Poor regulation of Th1 cytokine responses result in overstimulation of lymphocytes and macrophages, causing a severe, chronic inflammatory condition called delayed type hypersensitivity (DTH).[19] Interestingly, DTH reactions have recently been shown to be mediated by a novel Th cell species which expresses various isoforms of interleukin-17 (IL-17) that act as potent recruiters and activators of inflammatory cells such as neutrophils.[19] Other recently identified Th subsets have also found to be implicated in airway disease. For example, interleukin-9 (IL-9), a Th2-associated cytokine linked to diverse patterns of symptoms in allergies, is expressed by a unique Th cell type (Th9- helper T cells) that initiates cascades of inflammatory cell activity that contribute substantially to allergic tissue damage and symptoms.[20]

Strategies for characterising the nature of inflammatory illness must take into account these component processes while providing an easily understood comprehensive picture. In this study, we evaluated lymphocyte subpopulations in asthmatic patients, and healthy control subjects amongst the long-term residents of Kuwait, and compared the healthy group with healthy recent arrivals with the aim to assess any alteration in the fundamental patterns of immune reactivity among Kuwaitis in ways that affect pathogenesis of the disease. The data presented in this study does not provide evidence for involvement of any particular environmental toxicant in immune activation. However, it may serve as a basis for more comprehensive studies.

Material and Methods

Patients

Twenty-five non-smoking Kuwaiti patients, 14 males and 11 females, aged 15 to 32 years (mean 31.5 ± 2.3) diagnosed with bronchial asthma concurrent with rhinitis were included in this study. Their disease duration ranged from 1 to 10 years. All patients had been referred to the outpatient clinic of Al-Rashid Allergy Center, Sabah Hospital, Kuwait. Diagnosis of asthma was based on the guidelines proposed by the American Thoracic Society.[21] Diagnosis of allergic rhinitis was based on typical clinical symptoms (sneezing, running, itching and blocked nose) associated with sensitisation to at least one or more local pollen allergens for 2 or more consecutive seasons.[22,23] The diagnosis was confirmed by medical records of clinical symptoms for both asthma and rhinitis. In all patients, atopy was defined on the basis of one or more positive skin prick tests to the most common inhalant allergens (house dust mites + 3 most important local pollen allergens (Stallergens, France) and / or positive (> class II) of specific IgE to the same allergens. Mean serum IgE levels for asthmatic with rhinitis participants was 360 IU/ml (range, 185 to 450); specific IgE: class >2 of specifc IgE, for at least one of the above mentioned allergens. Forced expiratory volume in 1 second (FEV1) ranged between 60% and 80% in all allergic patients included in the study. The duration of disease ranged between 1 to 10 years. Patients were treated by inhaled corticosteroids (800 to 1200 μg/day), bronchodilators as needed, antihistamines (Cetirizine HCl 10 mg/day) and intranasal corticosteroids (Fluticason propionate 2 puffs/once daily) in rhinitis symptomatic patients. Each of the participants in this study were either newly diagnosed with both disorders and had not been prescribed medication; or had not taken cetirizine for 3 days and systemic corticosteroids for at least 6 weeks.

Two healthy control groups were included in this study. These included a group of 18 healthy Kuwaiti participants, 8 males and 10 females, aged 22 to 44 years (mean 25 ±

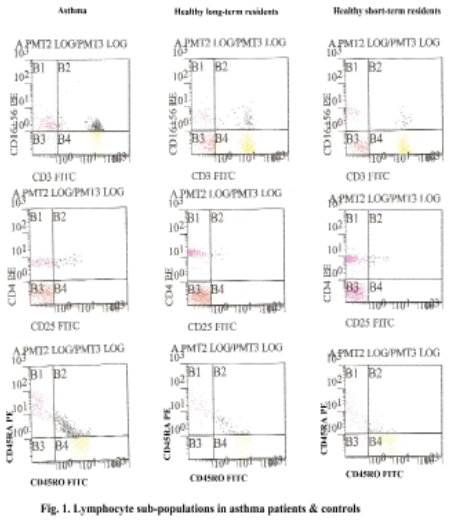

Fig. 1. Lymphocyte sub-populations in asthma patients & controls

Fig. 1. Lymphocyte sub-populations in asthma patients and controls. Percentage expression of CD3+CD56+ (NKT-like cells), CD4+CD25+ (T cells expressing IL-2R) and CD45RA/CD45RO dual expression on CD4+ T cells are shown for one subject from each group.

1.9 years). A second healthy group consisting of 10 non-Kuwaiti participants from Egypt and Syria: 4 males and 6 females aged 24 to 38 years (mean 27 ± 2.0 years) were also included. All the healthy subjects were non-allergic with negative personal and family history for atopic diseases. Total IgE levels for each subject were within the normal range (90 to 120 IU/ml), with negative specific IgE, normal value of pulmonary function tests and FEV1 >90%. All Kuwaiti subjects in this study were present in Kuwait during the 1991 and 2003 wars and were thus exposed to the full spectrum of toxicants present in the environment of the country during and after the conflict. The healthy non-Kuwaiti participants were newcomers to Kuwait, all of whom had been resident in the country between 3 to 6 months at the time of evaluation for this study. These individuals were included for comparison with healthy Kuwaitis. An informed consent agreement was obtained from all participants.

Lymphocyte Analysis

The amount of peripheral venous blood collected from each subject in EDTA tubes was 5 ml and analysed within 4 to 6 hours. Fifty µl of blood were incubated for 30 minutes at room temperature, together with 5 µl of flourescein-isothiocyanate (FITC), phycoerythrin (RD1) or PerCP (peridin chlorophyll protein) conjugated monoclonal antibodies (mAb), to surface markers of interest. The cells were then treated with Q-prep (Coulter Corporation, Hialeah, FL, USA) for haemolysis, stabilisation and amplification of the antigen-antibody reaction and fixation with paraformaldehyde. Two and 3 colour fluorescence analysis using an automated flow cytometer (Coulter Epics Altra) was performed. Positive analysis regions for cells expressing specific surface antigens were compared with isotypic controls and the specific binding of fluorophore-conjugated monoclonal antibodies was analysed according to standard methods recommended by the manufacturer. Monoclonal antibodies specific for human T lymphocytes (CD3+) and subpopulations were used which included the following cell types within the CD3+ compartment: CD3+CD56+ (NKT-like cells), CD4+CD25+^{high} (activated T cells with a presumed regulatory phenotype), CD4+CD25+^{low} (activated T cells with a presumed non-regulatory phenotype), CD4+HLA-DR+ (activated T cells), CD4+CD45RO+ (memory T cells), CD4+CD45RA+ (Naive T cells), CD4+CD29+ (helper/inducer T cells), CD4+CD54+ (ICAM1+ T cells), CD4+CD62+ (L-Selectin+ T cells), CD8+CD25+ (activated cytotoxic T cells), CD8+HLA-DR+ (activated cytotoxic T cells), CD8+CD38+ (activated cytotoxic T cells), CD4+CD212+ (IL-12 receptor β_1+ T cells). CD4+ T cells were analysed for dual expression of CD45RO/ CD45RA. All fluorophores were purchased from Immunotech, Coulter Corporation, Hialeah, FL, USA.

T lymphocytes with natural killer (NK) cell surface markers (phenotype and nomenclature): Human T cell subpopulations that express NK cell markers include CD3+CD56+ and CD3+CD16+CD56+.[24] At the time of this writing, no standard nomenclature for distinguishing these 2 immunophenotypes is universally agreed on and both are often referred to generically as "NKT" cells. Only the CD3+CD56+ phenotype was evaluated in blood of subjects in the present study and for the purpose of this report, these cells will be referred to as "NKT-like cells".

Statistical Analyses

Statistical analysis was performed using the independent t-test. All analyses were performed using the SPSS for Windows statistical package (Norusis/SPSS, Inc.). A value of P <0.05 was considered statistically significant.

Results

Tables 1 to 3 show the percentages of activated T cell phenotypes in peripheral blood of subjects with asthma and rhinitis in comparison to their levels in blood of healthy individuals evaluated in the present study. Both subject groups were recruited from among long-term residents of Kuwait, who had been present during the 1991 and 2003 Gulf wars and remained in-country until the time of participation in this investigation. In addition, a comparison was made between peripheral blood T cell phenotypes in healthy

Table 1. CD3+ T Lymphocyte Population and Subpopulations in Peripheral Blood of Asthma Patients versus Healthy Subjects

Lymphocyte Subset	Lymphocyte Subpopulation	Healthy subjects: recently arrived in Kuwait (n=10)	Healthy subjects: long-term residents of Kuwait (n = 18)	Asthmatic subjects:long-term residents of Kuwait (n = 25)
Pan T cells	CD3+	69.2 ± 3.5	73.2 ± 1.4	79.1 ± 2.2*
NK-like T cells	CD3+CD56+	3.7 ± 0.5	6.7 ± 1.5†	30.2 ± 3.5‡
NK cell subset	CD56+ CD16+	11.9 ± 1.6	11.6 ± 2.0	43.4 ± 3.9‡

Values (mean % ± SEM) are given as percentage of CD3+ T lymphocyte major population and sub-poplations.
Significance of group comparisons for asthma patients versus healthy subjects (long-term residents of Kuwait): *P <0.05, ‡P <0.001.
Significance of group comparisons for healthy long-term residents of Kuwait versus healthy short-term residents: †P <0.05.

Table 2. CD4+ T Lymphocyte Population and Subpopulations in Peripheral Blood of Asthma Patients versus Healthy Subjects

Lymphocyte Subset	Lymphocyte Subpopulation	Healthy subjects: recently arrived in Kuwait (n=10)	Healthy subjects: long-term residents of Kuwait (n = 18)	Asthmatic subjects: long-term residents of Kuwait (n = 25)
T helper cells	CD4+	37.3 ± 3.5	42.7 ± 2.1	47.1 ± 1.2*
T-helper cells expressing IL-2R	CD4+CD25+	5.9 ± 1.2	2.0 ± 0.6§	10.5 ± 2.5‡
Regulatory T-helper cells	CD4+CD25+^{high}	2.5 ± 0.6	0.8 ± 0.2§	1.5 ± 0.8
Activated T-helper cells	CD4+CD25+^{low}	3.2 ± 1.1	1.2 ± 0.4	9.7 ± 1.2‡
Activated T-helper cells	CD4+HLA-DR+	4.9 ± 1.6	4.4 ± 0.96	9.6 ± 1.3†
Memory T Cells	CD4+CD45RO+	54.4 ± 4.0	47.2 ± 4.4	75.0 ± 2.4‡
Naive T Cells	CD4+CD45RA+	42.4 ± 2.2	41.8 ± 1.9	48.6 ± 3.9
Naive-to-Memory Transitional	CD4+CD45RA+/CD45RO+	2.4 ± 1.6	8.6 ± 2.3§	23.6 ± 1.9‡
Helper/ inducer T cells	CD4+CD29+	30.6 ± 1.9	28.4 ± 2.4	55.2 ± 3.2‡
ICAM-1+ T-helper cells	CD4+CD54+	2.9 ± 0.4	1.9 ± 0.4	23.8 ± 4.5‡
L-Selectin+ T helper cells	CD4+CD62+	5.9 ± 3.5	8.2 ± 2.2	20.6 ± 4.1*
IL-12 receptor β_1+ Tcells	CD4+CD212+	0.71 ± 0.3	0.44 ± 0.25	23.1 ± 3.6‡

Values (mean % ± SEM) are given as percentage of CD4+ T lymphocyte major population and sub-populations.
Significance of group comparisons for asthma patients versus healthy subjects (long-term residents of Kuwait): *P <0.05, †P <0.01, ‡P <0.001.
Significance of group comparisons for healthy long-term residents of Kuwait versus healthy short-term residents: §P <0.05.

long-term residents and recent immigrants to the country.

Table 1 contains the results of the analysis of CD3+ subpopulations in all subjects. It shows a small but significant elevation in the overall size of the CD3+ T cell population (Pan-T cells) in asthmatic versus healthy long-term residents (P <0.05); and substantially greater representation by CD3+CD56+ (NKT-like cells); and CD56+CD16+ (NK) phenotypes in the asthmatic, versus healthy long-term resident group (P <0.001). Healthy, long-term residents of Kuwait exhibited representation of CD3+CD56+ (NKT-like cells) significantly greater than levels of these cells in recent immigrants (P <0.05), but no significant differences in representation of Pan T cells, or the NK sub-populations between the 2 groups (Table 1).

The analysis of CD4+ T cell subpopulations are given in Table 2 which shows that in comparison to healthy control subjects, peripheral blood of asthmatic, long-term residents of Kuwait contain significantly higher representation of all CD4+ subpopulations except for CD4+CD25+^{high} (regulatory T helper); and CD4+CD45RA+ (naive T) cells in a significance range of P <0.05 to P <0.001. Relative to recent immigrants, the healthy long-term residents exhibited lower levels of CD4+CD25+ and CD4+ CD25^{high} cell populations and higher levels of naive-to-memory transitional T cell populations (P <0.05).

Analysis of CD8+ subpopulation frequencies shown in Table 3 reveals a small but significant elevation in all CD8+ T cells in peripheral blood of asthmatic versus healthy long-term residents (P <0.05). In comparison to healthy, long-term residents, blood from asthmatic (long-term resident) subjects also contained significantly greater representation of the activated CD8+ T cell phenotypes CD8+CD25+ (P <0.01); and CD8+CD38+ (P <0.001), but no significant differences were noted between these 2 groups with respect to CD8+HLA-DR+ activated T cells (Table 3). Figure 1 shows lymphocyte subpopulation in each group.

Table 3. CD8+ T Lymphocyte Population and Subpopulations in Peripheral Blood of Asthma Patients versus Healthy Subjects

Lymphocyte Subset	Lymphocyte Subpopulation	Healthy subjects: recently arrived in Kuwait (n = 10)	Healthy subjects: long-term residents of Kuwait (n = 18)	Asthmatic subjects: subjects: long-term residents of Kuwait (n = 25)
Cytotoxic suppressor T cells	CD8+	27.9 ± 2.1	29.2 ± 1.5	33.5 ± 1.6*
Activated T-cytotoxic cells	CD8+CD25+	1.4 ± 0.7	0.94 ± 0.5	11.9 ± 2.8†
Activated T-cytotoxic cells	CD8+HLA-DR+	6.2 ± 0.7	10.3 ± 3.7	9.9 ± 1.1
Activated T-cytotoxic cells	CD8+CD38+	22.5 ± 3.2	18.6 ± 2.1	35.5 ± 2.7‡

Values (mean % ± SEM) are given as percentage of CD8+ T lymphocyte major population and subpopulations.
Significance of group comparisons for asthma patients versus healthy subjects (long-term residents of Kuwait): *P <0.05, †P <0.01, ‡P <0.001

Discussion

The present investigation demonstrates the correlation between lymphocyte subpopulation size representation and 2 major attributes of the subject population: occurrence of asthma concurrent with rhinitis; and time of residency in Kuwait relative to the 1991 and 2003 Gulf Wars. A major finding is that relative to healthy subjects, peripheral blood of those afflicted with asthma and rhinitis contain higher numbers of activated and memory T cells, a result expected, based on generally increased immune activation known to occur during the pathogenesis of asthma.[21,25,26]

The data failed to reveal existence of clear-cut correlation between general immune activation and duration of exposure to the wartime and postwar environment of Kuwait. There were nevertheless some intriguing observations suggesting that long-term residency in-country does indeed impact immune reactivity: Relative to healthy subjects recently arrived in-country, blood of healthy, long-term residents were observed to contain significantly elevated levels of NKT-like cells (CD3+CD56+) (P <0.05, Table 1); and naïve-to-memory transitional cells (CD4+CD45+RA/CD45RO) (P <0.05, Table 2).

The possible implications of elevated NKT-like cells in asthmatic versus healthy; and long-term versus short-term residents may be estimated based on the known immunoregulatory role of this cell population. T cells with NK surface markers express humoral factors that regulate inflammatory pathology in a wide range of immune responses including infections, anti-tumour responses, allograft rejection, graft-versus-host disease,[27-33] and autoimmunity.[18,34-36] These cells can also promote the polarization and differentiation of naive CD4 T cells into Th1 and Th2 immunophenotypes.[34] Despite the apparently protective role of T cells with NK markers in some pathologies,[33] previous investigations have shown that these cells adversely affect the prognosis of asthma, possibly through promotion of Th2-dependent airway damage.[37,38] For this reason, it is possible that occurrence and persistence of asthma and rhinitis may reflect the presence of elevated NKT-like cell representation in the test subject group and therapies that target these cells may contribute to resolution of asthma, rhinitis and other respiratory disease. The elevated levels of NKT-like cells observed in blood of asthmatics versus healthy individuals (P <0.001, Table 1) is consistent with the known pathogenic role of these cells in asthma and other airway disease.[37,38] Their elevated representation in blood of healthy long-term residents versus recent immigrants (P <0.05, Table 1) cannot be explained by the present data, however this phenomenon may reflect a yet-to-be-characterised etiologic factor in the environment of Kuwait acting to increase risk of airway disease for long-term residents.

A major objective in the present study was to identify relationships between occurrence of airway disease, or duration of exposure to the environment of Kuwait and CD4+CD25+^{high}, a presumed T cell phenotype regulatory phenotype (Treg) (CD4+CD25+^{low}, are presumed to be non-Treg activated T cells). Treg cells act as a host mechanism to reduce severity of inflammatory tissue damage in asthma and related disease by expression of humoral factors such as IL-10 at the site of airway tissue injury.[39,40] The role played by these cells as a critical host countermeasure to asthma, allergies and related syndromes is demonstrated by experiments in which depletion of Treg cells before allergen sensitisation of mice enhanced the severity of airway inflammation.[41] There is also strong evidence in humans that Treg cells inhibit pathogenic Th2 cell responses, suggesting that atopy can result from an imbalance between Th2 cells and Treg cells.[42] Interestingly, there are substantial decreases in the frequencies of allergen-specific IL-10-producing Treg cells, and increases in IL-4-producing T cells, in allergic individuals compared to healthy, nonatopic individuals.[43]

In the present study, the percentage representation of CD4+CD25+ activated T-helper cells in blood of asthmatic subjects was observed to be significantly elevated relative to healthy individuals (P <0.001, Table 2), which is consistent with the known role of activated T cells as a primary agent in the pathogenesis of asthma.[21,25] Interestingly,

CD4+CD25+^{high} (presumed Treg) cells were not significantly elevated in blood of subjects with asthma/rhinitis when compared with healthy controls (Table 2). A functional interpretation of this observation is that failure of the CD4+CD25+^{high} Treg population to expand in a protective response to asthma/rhinitis pathogenesis may contribute to disease severity.

The 3-fold lower percentage representation of CD4+CD25+^{high}, Treg cells in blood of healthy long-term residents versus healthy recent immigrants (P <0.05, Table 2) was intriguing since it reflected another difference in immunoregulatory capacity linked to duration of exposure to the environment of Kuwait. It is expected that reduced size of Treg populations systemically will increase the risk of a wide range of inflammatory disease, including but not limited to respiratory syndromes such as asthma and rhinitis. This hypothesis is speculative at the time of this writing, but it provides compelling incentive for intensive study of Treg populations in residents of Kuwait.

As shown in Table 2, the percentage of CD4+CD45RO+ memory T cells was found to be elevated in asthma patients relative to non-asthmatic control subjects, whereas no significant differences were seen between the 2 groups with respect to blood content of CD4+CD45RA+ naive T cells. Since the transition of naïve to memory CD45 isoforms is known to occur as a result of T cell response to antigenic stimulation,[44,45] an interpretation that can be made of this finding is that a disease-associated antigen exposure occurred. However, the scope of this study does not allow any information regarding nature of such an antigen and its relationship to asthma pathogenesis to be extracted from the data. Of particular interest is the elevated frequency in patient's blood, of T cells bearing both naive and memory markers: CD4+CD45RA+RO+, which is known to be a naive-to-memory transitional phenotype seen shortly following antigenic exposure.[45] Expanded populations of these cells have been reported in some autoimmune diseases[46] and may eventually provide insight into how environmental factors contribute to allergy and asthma.

The present study was conducted to begin characterisation of patterns of immune reactivity among Kuwaitis afflicted with asthma and allergic disorders. The toxic burden to which residents of this country were subjected following the 1991 and 2003 Gulf Wars may have resulted in immunologic features not previously reported for these diseases. Significantly higher frequencies of NKT-like cells and CD4+CD45RA+/CD45RO+ (naive-memory transitional) (P <0.05) but lower levels of CD4+CD25^{high} T (presumed regulatory) cells (P <0.05) were observed in long-term residents of Kuwait relative to healthy recently arrived individuals (Tables 1 and 2). These results suggest that long-term residents of Kuwait exhibit elevated levels of immune activation and reduced immunoregulation relative to recent arrivals. This could presumably contribute to increased risk of disease via enhanced triggering of allergic reactions.

In conclusion, the findings reported here provide a profile of T cell activation in a sample population of Kuwaiti residents afflicted with asthma and rhinitis. Other than providing a profile of peripheral blood T cell activation patterns in the patients versus control subjects, the results of this study raise some interesting questions. For instance, how do expression levels of CD4+CD45+RA+/RO+ naive-to-memory transitional phenotypes in Kuwaiti patients compare to persons afflicted with similar disorders in other parts of the world? Presence of these cells at high frequencies, suggest ongoing antigen exposure. If this is indeed the case, what is the nature of the antigen? It is also worthwhile to consider the relative levels of NKT-like and regulatory cell (CD4+CD25^{high}) frequencies in Kuwaiti versus other populations in terms of the capacity of each phenotype to promote or suppress symptoms of asthma and allergic disease. Ongoing and future investigations will build on these results to provide a clear picture of how pathogenesis of these disorders among the indigenous population of the Arabian Gulf region may have evolved unique characteristics.

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