

Diminution of T cells with $\gamma\delta$ receptor in the peripheral blood of allergic asthmatic individuals

K.-S. CHEN*, K. H. MILLER† & D. HENGHOLD#

Procter & Gamble Pharmaceuticals Research Division, *Beauty Care Technology, Miami Valley Laboratories and
†Biometrics and Statistical Sciences Department, Regulatory and Clinical Development Division, #Procter and Gamble
Company, Miami Valley Laboratories, Cincinnati, Ohio, USA

Summary

Background T cells are shown to regulate allergy and asthma. They are heterogeneous by virtue of surface receptor subtypes ($\alpha\beta$ and $\gamma\delta$ receptors), however, their pathophysiological roles in asthma are unclear.

Objective and methods The present study employed flow cytometric analysis to compare the size of T-cell subsets and eosinophils present in the peripheral blood of healthy, allergic and allergic asthmatic individuals.

Results Current results demonstrated that the percentages of $\gamma\delta$ T cells declined from 4.1% in healthy to 3.2% in the allergic subjects and to a significantly lower ($P=0.01$) 2.4% in allergic asthmatics. The absolute numbers of circulating $\gamma\delta$ T cells also were diminished in a similar fashion such that healthy individuals had a significantly higher mean cell count ($91.8 \times 10^3/\text{mL}$) than did allergic asthmatics ($47.8 \times 10^3/\text{mL}$) ($P=0.0266$). In contrast, $\alpha\beta$ T cells were comparable in the healthy, allergic, and allergic asthmatic populations (65.3%, 65.8% and 69.4%, respectively); the differences were not statistically significant. On a populational basis, the proportion of individuals having both $\gamma\delta$ T cells $\leq 4.1\%$ and eosinophils $\geq 2.1\%$ was the lowest in the healthy population (30.8%), but was elevated in the allergic group (85.7%) as well as in the allergic asthmatic group (86.6%). However, on an individual basis, those who had reduced $\gamma\delta$ T cells did not have consistently higher eosinophil counts or IgE level.

Conclusion It was concluded that no significant correlation existed between the levels between $\gamma\delta$ T cells and eosinophils or between $\gamma\delta$ T cells and IgE present in the peripheral blood. This report, for the first time, documents that allergic asthmatics have reduced $\gamma\delta$ T cells with reciprocally elevated eosinophil numbers in their peripheral circulation. However, it does not indicate that the reduction of $\gamma\delta$ T cells directly correlates with the predominance of eosinophils or IgE levels in the diseased populations. The pathophysiological role of $\gamma\delta$ T cells in allergic diseases awaits further exploration.

Keywords: allergy, asthma, eosinophil, flow cytometry, IgE, lymphocyte, rhinitis, $\gamma\delta$ T cell

Clinical and Experimental Allergy, Vol. 26, pp. 295–302. Submitted 20 December 1994; revised 1 August 1995; accepted 6 September 1995.

Introduction

In the peripheral blood of healthy human subjects, 5–10% of T cells express $\gamma\delta$ T-cell receptors (TCR) [1].

Correspondence: K.-S. Chen, Procter & Gamble Pharmaceutical Research Division, Miami Valley Laboratories, Procter & Gamble Company, PO Box 538707, Cincinnati, Ohio 45253-8707, USA.

$\gamma\delta$ T cells are predominantly CD4^- and CD8^- with occasional CD8^+ and rare CD4^+ expression. Depending on the diversity of $\gamma\delta$ TCR, utilization of specific γ and δ gene segments and location, $\gamma\delta$ T cells are subdivided into two populations: one is associated with the skin [2, 3], tongue, and the epithelial mucosa of vagina, uterus [4], and expresses distinct but homogenous TCR [4]; the

second population localizes to blood, peripheral lymphoid tissue and gut [5] and is generated later in ontogeny with expression of more diverse TCR [6]. The biological function of $\gamma\delta$ T cells is poorly understood.

$\gamma\delta$ T cells lack significant involvement in classical T-cell responses but are detected in markedly elevated levels in certain pathological conditions such as bacterial [7, 8], parasitic [9] and viral infections [10–13], joint inflammation [14, 15], autoimmune responses such as coeliac disease [16, 17] and eosinophilic granuloma [18]. The role of $\gamma\delta$ T cells in the induction and/or maintenance of inflammatory responses remain speculative. $\gamma\delta$ T cells and $\alpha\beta$ T cells secrete a similar profile of cytokines, express similar activation and adhesion molecules, and are able to lyse antigen-bearing target cells [19, 20]. Ontogenically, the appearance of $\gamma\delta$ T cells precedes $\alpha\beta$ T cells. They home to distinct sites, such as epithelial tissues and express a more limited repertoire of TCR variable gene, coincidental with limited junctional diversity [6, 21]. Antigenic recognition by $\gamma\delta$ T cells is mostly unrestricted by MHC and skews toward the recognition of bacterial antigens, heat-shock proteins, or MHC class Ib [22–26] whereas $\alpha\beta$ T cells are MHC-restricted in the antigenic recognition.

In allergy and extrinsic asthma, the IgE level is markedly increased in the peripheral circulation [27]. Eosinophils are regarded as important proinflammatory effector cells in asthma, and are also elevated in the blood circulation, airway tissues and bronchoalveolar lavage fluids [28–32]. The function, survival and priming of eosinophils [33, 34] is regulated by cytokines such as IL 5, a product of CD4⁺ T cells and eosinophils [35]. Since T cells are pivotal in the pathogenesis of allergy-associated illness [36, 37], this study explored whether there is a change of T-cell subsets present in the peripheral blood of allergic and allergic asthmatic individuals or not. This study further examined the regulatory relationship of $\gamma\delta$ T cells to two illness-associated components (eosinophils and IgE) important in the pathogenesis of allergy and/or allergic asthma.

Materials and methods

Subjects

Twenty-three healthy adult volunteers (eight men and 15 women) ranging from 20 to 54 (mean 33) years who had no prior history of respiratory allergy or asthma, had experienced no recent illness and were not under any medication. All, except one individual, were non-smokers. Twenty-two allergic non-smokers (seven men, 15 women) between 23 and 51 (mean 37) years were previously verified as having allergic reactivities with skin-prick

tests by physicians and experienced varying degrees in severity of hay fever symptoms at the time of study. Among 22 participants, four did not take any medication while nine received a single medicine (antihistamine or bronchodilator) only. The remainder used two or three therapeutic agents (antihistamine, inhaled steroid, desensitizing injections) concurrently for symptomatic relief. Seventeen allergic asthmatic volunteers (three men, 14 women) ranging from 23 to 51 (mean 39) years were previously confirmed as having both allergy and asthma by physicians. Of the 17 extrinsic asthmatics, one individual did not use any medicine, three only used antihistamine whereas six individuals received combined therapy with two drugs, four with three drugs, and three with four drugs from antihistamine, inhaled steroid, bronchodilator, or desensitizing injections. One individual was a smoker and the remainders were non-smokers. All participants gave their informed consent to this study.

Blood collection

Peripheral blood was collected aseptically by venipuncture from each individual into a sterile K₃ EDTA Vacutainer tube (Becton Dickinson, New Jersey, USA) and a sterile SST brand Vacutainer (Becton Dickinson) for serum separation by centrifugation. Sera were stored at -20°C for total IgE quantitation. Fresh peripheral blood from each individual was immediately analysed by the Hematology Analyser and also stained with a battery of commercial reagents (Becton Dickinson, San Jose, CA, USA) specific to various cellular populations for flow cytometric analysis.

Reagents

Monoclonal antibodies (MoAb) labelled with either fluorescein isothiocyanate (FITC) or phycoerythrin (PE) were purchased from Becton Dickinson Immunocytometry Systems: anti-Leu-4 (CD3) FITC, anti-Leu-3a (CD4) FITC, anti-Leu-2a (CD8) PE, anti-Leu12 (CD19) PE, anti-TCR α/β -1 FITC, anti-TCR γ/δ -1 PE, SimultestTM γ 1 FITC/ γ 1 PE control, and Simultest LeucoGATETM (Anti-HLe-1 FITC + Anti-Leu-M3 PE). The stock solution of FACS[®] Lysing solution (Becton Dickinson) was diluted 10-fold with reagent-grade water to be the working solution. Hank's balanced salt solution (HBSS; Gibco/BRL, Grand Island, NY, USA) was used for all washing steps and for the final dilution of single cell suspensions.

Cellular staining

Whole blood samples were first stained with the immunophenotyping reagent, γ 1 FITC/ γ 1 PE (a negative control),

to determine the level of background staining, and then with LeucoGATE™ to determine the lymphocyte gate. Briefly, 100 μ L of blood was delivered into 12 $\times$ 75 mm polystyrene test tubes (Falcon, Franklin Lakes, NJ, USA). Twenty microlitres of antibody was added to the appropriate tube to create direct two-colour immunofluorescent staining. After 20 min incubation in the dark at room temperature, 2 mL of FACS solution was added and the cells were incubated for an additional 10 min. Cells were washed by HBSS and by centrifuga-

tion at 2000 rpm for 10 min at 4°C and the cell pellets were washed again with HBSS. Each tube was resuspended with 1 mL of HBSS and was immediately analysed on a FACScan™ flow cytometer (Becton Dickinson) equipped with a 488 nm argon ion laser.

Flow cytometric acquisition and analysis

The FACScan was optimized using CaliBRITE beads (Becton Dickinson) and AutoCOMP software to set

Table 1. Percentages of cellular populations and serum IgE titers in the peripheral blood of the healthy, allergic, and allergic asthmatic individuals

Study subject	Study population and their percentages			Overall <i>F</i> -test <i>P</i> -value ^a	Pairwise contrasts (<i>P</i> -value) ^b
	Healthy	Allergic	Allergic/asthmatic		
	Mean $\pm$ SEM	Mean $\pm$ SEM	Mean $\pm$ SEM		
CD3 ⁺ T cells	69.8 $\pm$ 1.62 22* (66.6, 73.0)**	69.6 $\pm$ 2.22 22 (65.3, 74.0)	72.7 $\pm$ 1.65 17 (69.5, 75.9)	0.48	—
CD4 ⁺ T cells	41.6 $\pm$ 1.64 23 (38.4, 44.8)	41.6 $\pm$ 2.34 22 (37.0, 46.2)	47.2 $\pm$ 2.42 17 (42.5, 52.0)	0.13	—
CD8 ⁺ T cells	31.3 $\pm$ 1.29 23 (28.8, 33.9)	32.0 $\pm$ 1.53 22 (29.0, 35.0)	28.2 $\pm$ 1.73 17 (24.8, 31.6)	0.21	—
$\alpha\beta$ T cells	65.3 $\pm$ 1.68 23 (62.0, 68.6)	65.8 $\pm$ 2.32 22 (61.3, 70.4)	69.4 $\pm$ 1.76 17 (65.9, 72.8)	0.33	—
$\gamma\delta$ T cells ^c	4.1 $\times$ / $\div$ 1.16 23 (1.8, 9.4)	3.2 $\times$ / $\div$ 1.16 22 (1.4, 7.3)	2.4 $\times$ / $\div$ 1.11 17 (1.1, 5.2)	0.04	H > A/A (<i>P</i> = 0.01)
Eosinophils ^c	2.1 $\times$ / $\div$ 1.10 23 (1.0, 4.6)	2.9 $\times$ / $\div$ 1.15 22 (1.3, 6.4)	4.0 $\times$ / $\div$ 1.15 17 (1.8, 9.0)	0.005	H < A/A (<i>P</i> = 0.001)
IgE (IU/mL) ^c	31.2 $\times$ / $\div$ 1.32 21 (12.1, 80.5)	54.4 $\times$ / $\div$ 1.29 22 (21.5, 137.9)	109.4 $\times$ / $\div$ 1.40 16 (40.0, 299.6)	0.02	H < A/A (<i>P</i> = 0.004)

^a All one-way analysis of variance (ANOVA) results were corroborated by the Kruskal–Wallis test at the 0.05 level of significance; pairwise tests were corroborated with the Wilcoxon rank-sum test.

^b Pairwise contrasts by Fisher's Protected LSD were performed only where the Overall *F*-test *P*-value $\leq$ 0.05; contrasts were also declared significant only for *P*-value $\leq$ 0.05.

^c Statistical analyses were performed on log transformed data to satisfy variance requirements necessary to perform an analysis of variance (ANOVA) and the calculation of their geometric mean values.

* The numerical number displayed in the mid-range denotes the number of study subjects.

** The numbers in the parenthesis denote 95% confidence intervals.

photomultiplier voltages, to adjust fluorescence compensation, and to check the detector's sensitivity following the manufacturer's procedures. A total of 15 000 events was collected from each sample and data were acquired in list mode using LYSYS II software. A lymphocyte light-scatter gate was defined using the Simultest LeucoGATE™ reagent. The gate was used to determine percentage of stained lymphocytes. Fluorescence intensity markers were set using the γ 1 FITC/ γ 1 PE isotype controls as an indication of non-specific staining.

Serum IgE quantitation

Serum samples were blind-coded prior to the quantitation of the total IgE contents and the titres were expressed by IU/mL. Tests were performed by National Reference Laboratory (Louisville, KY, USA) using the Ciba Corning Automated Chemiluminescence System (Ciba Corning Diagnostic Corp., Medfield, MA, USA).

Differential analysis of blood cell subpopulations

Differential leucocyte counts were determined by both FACScan using the LeucoGATE™ reagents and a Technicon H-le Hematology Analyser (Miles Laboratories, Tarrytown, NY, USA).

Statistical analysis

To test for differences between the healthy, allergic, and allergic asthmatic populations (Table 1), a one-way analysis of variance (ANOVA) was first performed for each cell type. If a significant difference was detected among the three populations via a F-test, then pair-wise comparisons were conducted on the adjusted least squares means (Fisher's Protected LSD). Results for each cell type were respectively corroborated with the Kruskal-Wallis test for the overall populational effect and with Wilcoxon rank sum tests for pairwise comparisons between populations on a given cell type. All statistical tests were conducted at the $P = 0.05$ level of significance.

Residual analyses were performed on the residual error terms of the one-way ANOVAs in order to evaluate the validity of assumptions inherently made in the models. In order to stabilize the variances among the populations, it was necessary to log-transform the following three elements ($\gamma\delta$ T cells, eosinophils, and IgE) before performing any analyses.

Correlation analyses were conducted, among the cell types where significant population differences were observed, to explore possible linear relationships. For each population, Spearman correlation coefficients and

their corresponding P -values were calculated to detect if observed relationships were significant.

Results

T-cell subsets

Peripheral blood T cells carrying $\alpha\beta$ or $\gamma\delta$ TCR were analysed by flow cytometry. $\alpha\beta$ T cells from 23 healthy

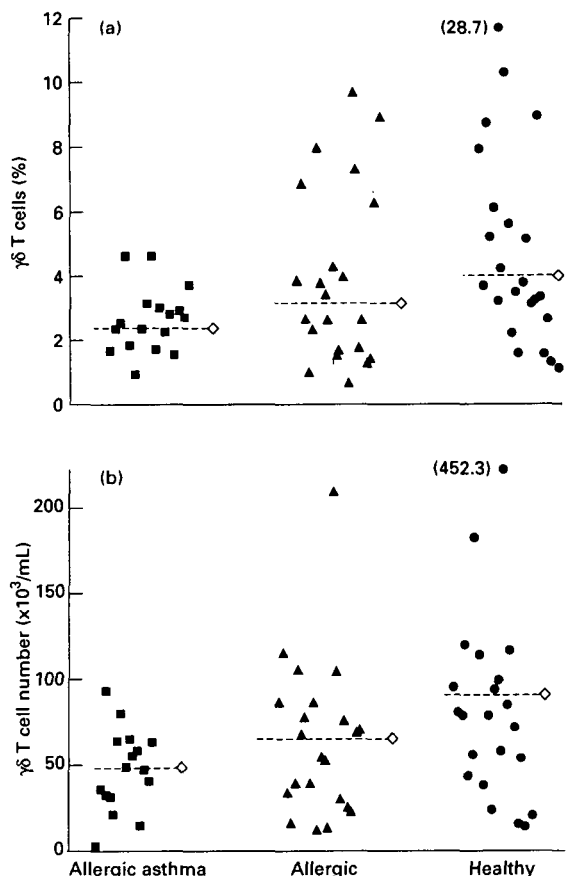


Fig. 1. Distribution of circulating $\gamma\delta$ T cells, in percentage (a) and in absolute number (b), present in the allergic asthmatic, allergic, and healthy populations. Each dot indicates the percentage (1-a) and absolute number (1-b) of $\gamma\delta$ T cells of a single individual in the designated (■, allergic asthmatic; ▲, allergic; ●, healthy) study populations. The geometric mean values ($- - \diamond$) $\pm$ standard errors in percentage of $\gamma\delta$ T cells (1-a) for allergic asthmatic, allergic and healthy groups were: $2.4 \times / \div 1.1$; $3.2 \times / \div 1.2$; and $4.1 \times / \div 1.2$, respectively. Similarly, the geometric mean values $\times / \div$ standard errors for the absolute number of $\gamma\delta$ T cells (1-b) in the corresponding study groups were: $47.8 \times 10^3 \pm 6 \times 10^3/\text{mL}$; $65.7 \times 10^3 \pm 9.7 \times 10^3/\text{mL}$; and $91.8 \times 10^3 \pm 19.2 \times 10^3/\text{mL}$, respectively. Both the percentage and cell number in allergic asthmatics are significantly ($P = 0.01$ and 0.026 , respectively) lower than those in the healthy group.

individuals had an arithmetic mean of 65.3% or $1.2 \times 10^6/\text{mL}$. The profiles for 22 allergics and 17 allergic asthmatics and their means in percentages or in cell numbers were 65.8% or $1.2 \times 10^6/\text{mL}$ and 69.4% or $1.3 \times 10^6/\text{mL}$, respectively, were comparable to the healthy population and to each other (Table 1). On the contrary, $\gamma\delta$ T cells from the peripheral blood of healthy donors had a geometric mean value of 4.1% with a distribution ranging from 1.2 to 28.7% (Fig. 1a). Allergic individuals had $\gamma\delta$ T cells between 0.8% and 9.8% with a geometric mean of 3.2% whereas allergic asthmatics had a significantly lower geometric mean of 2.4% ($P = 0.01$, Table 1 and Fig. 1a) and a range spanning between 0.9% and 4.6% (Fig. 1a). A similar differential pattern exists in the cell number of circulating $\gamma\delta$ T cells among these populations: $91.8 \times 10^3/\text{mL}$ (healthy group); $65.7 \times 10^3/\text{mL}$ (allergic individuals); and $47.8 \times 10^3/\text{mL}$ (allergic asthmatics) (Fig. 1b). Only allergic asthmatics had a significantly lower mean number of $\gamma\delta$ T cells than in healthy individuals ($P = 0.0266$). In the healthy group, 13 out of 23 (56.5%) individuals had $\gamma\delta$ T cells equal to or lower than the healthy population geometric mean of 4.1% while 14 of 22 (63.6%) allergic and 15/17 (88.2%) allergic asthmatic individuals fell in this lower range (Fig. 1a). The predominance rates of $\text{CD}3^+$, $\text{CD}4^+$ or $\text{CD}8^+$ T cells were comparable among the three study groups, except for $\text{CD}4^+$ cells which were slightly elevated among allergic asthmatics (Table 1).

Serum IgE

The geometric mean concentration of total IgE in the sera of healthy individuals was 31.2 IU/mL while there was a 74% increase to 54.4 IU/mL in the allergics and a significant 250% increase ($P = 0.004$) to 109.4 IU/mL in the allergic asthmatics (Table 1).

Blood eosinophil counts

In the healthy population, eosinophils ranged from 0.9% to 4.8% with a geometric mean of 2.1% (Fig. 2). In the allergic population, eosinophils ranged from 1.0% to 10.6% with a higher geometric mean value of 2.9% (Fig. 2); further, 15 out of 22 (68.2%) individuals had counts equal to or greater than the geometric mean for the healthy group (2.1%). The allergic asthmatic population with a range of 1.1–8% eosinophils had a significantly higher ($P = 0.001$) geometric mean value of 4% (Table 1) compared with healthy individuals; further, 14 out of 17 adults (82.4%) were $\geq 2.1\%$ (Fig. 2). Similarly, the actual cell number of eosinophils in the peripheral blood of allergic asthmatic population ($75.2 \times 10^3/\text{mL}$), was observed to be higher than allergic individuals

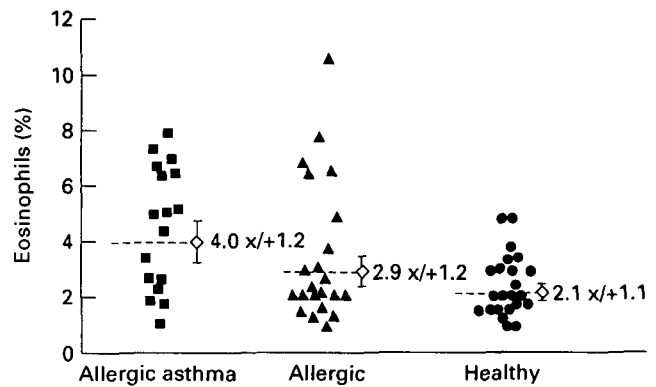


Fig. 2. Distribution of circulating eosinophils present in the allergic asthmatic, allergic, and healthy populations. Each dot represents the percentage of eosinophils of a single individual in designated (■, allergic asthmatic; ▲, allergic; ●, healthy) study populations. The geometric mean values (---◇) $\times/\div$ standard error of each study group were indicated.

($60.9 \times 10^3/\text{mL}$), is significantly ($P = 0.0033$) higher than in the healthy individuals ($41.8 \times 10^3/\text{mL}$).

Relationships among $\gamma\delta$ T cells, eosinophils and IgE

To assess whether there was a relationship between $\gamma\delta$ T cells and eosinophils, correlation analyses were performed in each population. Spearman's correlation coefficients ($r = -0.14$, $P = 0.58$) did not show the level of $\gamma\delta$ T cells of an individual correlated with his own level of eosinophils within the allergic asthmatic group. Neither did the identical assessments indicated a linear relationship between $\gamma\delta$ T cells and eosinophils in individuals within the allergic ($r = -0.06$, $P = 0.78$) or healthy groups ($r = 0.19$, $P = 0.38$). Thus, individuals with higher levels of eosinophils in any study groups did not significantly correlate to lower counts of $\gamma\delta$ T cells.

In the allergic asthmatic population, the level of serum IgE proportionately corresponded to the level of circulating eosinophils ($r = 0.49$, $P = 0.05$). However, in the allergic ($r = 0.34$, $P = 0.12$) and healthy populations ($r = 0.22$, $P = 0.33$), no linear relationship between IgE and eosinophils could be established. By correlation analysis, the levels of $\gamma\delta$ T cells were not significantly correlated with the level of serum IgE in individuals in any of the three study populations.

Discussion

This study shows, for the first time, that both the percentage and absolute number of $\gamma\delta$ T cells in the peripheral blood of individuals with allergic asthma are significantly

reduced which are contrary to their elevation of eosinophils and IgE.

Both allergic and allergic asthmatic populations had an elevated serum total IgE concentration that is consistent with others [38]. Eosinophils as well as T cells are considered as the key cell types responsible for the pathogenesis of respiratory allergy and allergic asthma [36,37]. Eosinophils in these diseased individuals are elevated both in circulation [28, 30, 31] and in bronchoalveolar lavage fluids [29]. Our present observation of elevated eosinophils in the peripheral blood of allergic and allergic asthmatic populations are consistent with others [28,30,31]. Activation and recruitment of eosinophils is modulated by cytokines such as interleukin-5 (IL-5) and granulocyte macrophage-colony stimulating factor (GM-CSF) that are produced by T cells and other cell types [33]. CD4⁺ T cells [39] and eosinophil [34,35] produce IL-5 that regulates the function, survival and priming of eosinophils and ultimately leads to eosinophilic degranulation and tissue damage [33,34]. The prevalence of circulating CD4⁺ T cells in allergic individuals did not significantly change but had a directional increase from 41.6% to 47.2% in the allergic asthmatics. At the present time, it is impossible to speculate on the biological significance of an elevated CD4⁺ T-cell population solely by the surface marker. The distribution profile of $\gamma\delta$ T cells from 23 healthy individuals scattered from 1.2% to 28.7% with a mean of 4.1% which is in agreement with the normal range previously reported in the healthy population [1]. Thirteen out of 23 healthy individuals had $\gamma\delta$ T cells equal to or below this populational mean value. The population of allergic asthmatics whose $\gamma\delta$ T-cell level was $\leq 4.1\%$ was 88.2%, thus clearly demonstrating a reduced $\gamma\delta$ T-cell population in allergic asthma. $\gamma\delta$ T cells were reduced not only in percentage in allergic asthmatic but also the absolute cell number in the peripheral blood was significantly diminished by twofold in comparison to the healthy individuals. It is possible that the reduction in $\gamma\delta$ cells in the peripheral circulation is the consequence of their enhanced migratory capability through endothelium [40] to the inflamed respiratory tract. This hypothesis awaits further exploration on the prevalence rate of $\gamma\delta$ T cells in the nasal and lung lavages of allergic and extrinsic asthmatic patients, although a single report fails to find a change in the populational size of $\gamma\delta$ T cells present in the bronchoalveolar lavage fluids from asthmatics [41]. Since the presence of an increased number of activated eosinophils has been suggested to be pathogenic in asthma, we examined if a high level of peripheral blood eosinophils was related to a reduced population of $\gamma\delta$ T cells. On the populational basis, there was an inverse relationship of an elevated eosinophil for a reduced $\gamma\delta$ T cells, and it also corroborated with the

disease progression in severity from healthy to allergy to allergic asthma. However, subsequent correlation analysis on a single individual basis failed to establish that individuals having lower number of $\gamma\delta$ T cells had proportionally higher counts of eosinophils. This finding favoured the conclusion that $\gamma\delta$ T cells are not a factor driving changes in the eosinophil population.

It has been demonstrated that $\gamma\delta$ T cells in lungs of mice recovering from influenza virus infection undergo a populational shift from $\gamma 4$ to $\gamma 2/\gamma 1$ phenotype [11] and that there is a further heterogeneity of $\gamma\delta$ T cells based on the types of cytokines produced [19,20]. $\gamma\delta$ T cells can be further subdivided into either CD4⁺ or CD8⁺ surface marker, as seen in the inflamed gingiva tissue, or bear CD4 and CD8 double-negative markers present in the healthy mucosa tissue; they show different cytokine secretion profile [21]. We did not identify the surface markers of $\gamma\delta$ T cells in the present study and thus could not ascribe their biological influences. It is conceivable, however, that $\gamma\delta$ T cells or subsets may thereof indirectly modulate the formation and/or biological activity of eosinophils or other regulatory cell types responsible for the pathogenic process. Future investigation on the surface markers of $\gamma\delta$ T cells in circulation as well as nasal and lung lavages from allergic patients may shed light on their role in modulating the pathogenesis of allergy and/or asthma.

$\alpha\beta$ T cells bear CD4 or CD8 phenotype and are the major regulatory cells in the synthesis of IgE via their secreted cytokines, such as IL-4, IL-5, and IFN γ . Recently, $\gamma\delta$ T cells are reported to support IgE synthesis in mice that are congenitally deficient in $\alpha\beta$ T cells [42]. Furthermore, $\gamma\delta$ T cells are shown to suppress the expansion of TH₂ CD4⁺ T cells [43] which upregulate the IgE formation. Therefore, a reduced level of $\gamma\delta$ T cells may enhance IgE synthesis. In addition, $\gamma\delta$ T cells secrete cytokines such as IFN γ [20,44], IL-4, and IL-5 [19] that closely regulate IgE synthesis by B cells. Lastly, $\gamma\delta$ T cells possess the high affinity receptor for IgE (Fc ϵ RI) while $\alpha\beta$ T cells do not [45]. Therefore, these collectively lead to the speculation that the diminished $\gamma\delta$ T cells may directly or indirectly influence the immunoregulatory networks, in addition to $\alpha\beta$ T cells, involved in the IgE formation.

So far, all putative linkages of $\gamma\delta$ T cells to modulate pathogenesis of infectious diseases [7–13] and inflammatory diseases [14–17] are attributed by their expanded population in the target tissues. In contrast, this report documents a diminished $\gamma\delta$ T cells are potentially associated with inflammatory diseases of the respiratory tract, such as allergy and extrinsic asthma. The differential regulatory mechanism by $\gamma\delta$ T cells in these diseases awaits future elucidation.

The present demonstration of diminished $\gamma\delta$ T cells in the peripheral blood may be attributed by their migration to the respiratory tract of the allergics and the allergic asthmatics or the suppression by other immune modulatory cells and/or their secreted mediators. In order to ascertain the pathophysiological role of $\gamma\delta$ T cells in respiratory allergy and asthma, future investigations on their predominance and phenotypic distributions in both nasal and lung lavage fluids may shed more light on their regulatory activities in the disease pathogenesis.

Acknowledgements

The authors are indebted to the medical assistance of N. L. Martin, E. Davis, J. Harrod, D. Myles for drawing blood samples and Mr G. W. Dameron for differential cell analysis. The authors also appreciated the critical review of the manuscript by Dr M. K. Robinson.

References

- 1 Lanier LL, Federspiel NA, Ruitenberg JJ et al. The T cell antigen receptor complex expressed on normal peripheral blood CD4⁺, CD8⁺ T lymphocytes. A CD3-associated disulfide-linked gamma chain heterodimer. *J Exp Med* 1987; 165:1076–94.
- 2 Asarnow DM, Kuziel WA, Bonyhadi M et al. Limited diversity of $\gamma\delta$ antigen receptor genes of Thy-1⁺ dendritic epidermal cells. *Cell* 1988; 55:837–47.
- 3 Koning F, Stingl G, Yokoyama WM et al. Identification of a T3-associated gamma delta T cell receptor on Thy-1⁺ dendritic epidermal cell lines. *Science* 1987; 236:834–7.
- 4 Itohara S, Farr AG, Lafaille JJ et al. Homing of a $\gamma\delta$ thymocyte subset with homogeneous T-cell receptors to mucosal epithelia. *Nature* 1990; 343:754–7.
- 5 Goodman T, Lefrancois L. Expression of the $\gamma\delta$ T-cell receptor on intestinal CD8⁺ intraepithelial lymphocytes. *Nature* 1988; 333:855–8.
- 6 Raulet DH. The structure, function, and molecular genetics of the $\gamma\delta$ T cell receptor. *Annu Rev Immunol* 1989; 7:175–207.
- 7 Munk ME, Gatrill AJ, Kaufmann SH. Target cell lysis and IL-2 secretion by $\gamma\delta$ T lymphocytes after activation with bacteria. *J Immunol* 1990; 145:2434–9.
- 8 O'Brien RL, Happ MP, Dallas A et al. Stimulation of a major subset of lymphocytes expressing T cell receptor $\gamma\delta$ by an antigen derived from *Mycobacterium tuberculosis*. *Cell* 1989; 57:667–74.
- 9 Rosat JP, MacDonald HR, Louis JA. A role for $\gamma\delta$ T cells during experimental infection of mice with *Leishmania major*. *J Immunol* 1993; 150:550–5.
- 10 Doherty PC, Allan W, Eichelberger M et al. Involvement of $\gamma\delta$ T cells in respiratory virus infections. *Current Top Microbiol Immunol* 1991; 173:291–6.
- 11 Carding SR, Allen W, Kyes S et al. Late dominance of the inflammatory process in murine influenza by $\gamma\delta$ ⁺ T cells. *J Exp Med* 1990; 172:1225–31.
- 12 Johnson RM, Lancki DW, Sperling AI et al. A murine CD4⁺, CD8⁺, T cell receptor- $\gamma\delta$ T lymphocyte clone specific for herpes simplex virus glycoprotein I. *J Immunol* 1992; 148:983–8.
- 13 Sciamma R, Johnson RM, Sperling AI et al. Unique antigen recognition by a herpesvirus-specific TCR- $\gamma\delta$ cell. *J Immunol* 1994; 152:5392–7.
- 14 Smith MD, Broker B, Moretta L et al. T $\gamma\delta$ cells and their subsets in blood and synovial tissue from rheumatoid arthritis patients. *Scand J Immunol* 1990; 32:585–93.
- 15 Söderström K, Bucht A, Halapi E et al. High expression of V γ 8 is a shared feature of human $\gamma\delta$ T cells in the epithelium of the gut and in the inflamed synovial tissue. *J Immunol* 1994; 152:6017–27.
- 16 Rust C, Kooy Y, Pena S et al. Phenotypical and functional characterization of small intestinal TCR $\gamma\delta$ ⁺ T cells in coeliac disease. *Scand J Immunol* 1992; 35:459–68.
- 17 Trejdosiewicz LK, Calabrese A, Smart CJ et al. $\gamma\delta$ T cell receptor-positive cell of human gastrointestinal mucosa: occurrence and V region gene expression in *Helicobacter pylori*-associated gastritis, coeliac disease and inflammatory bowel disease. *Clin Exp Immunol* 1991; 84:440–4.
- 18 Shijubo N, Shigehara K, Tsutahara S, Abe S. Increased level of circulating $\gamma\delta$ T cells in a patient with eosinophilic granuloma. *Chest* 1994; 105:967–8.
- 19 Christmas SE. Cytokine production by lymphocytes bearing the gamma-delta T-cell antigen receptor. *Chem Immunol* 1992; 53:32–46.
- 20 Bluestone JA, Cron RQ, Barrett TA et al. Repertoire development and ligand specificity of murine TCR $\gamma\delta$ cells. *Immunol Rev* 1991; 120:5–33.
- 21 Tonegawa S, Berns A, Bonneville M et al. Diversity, development, ligands, and probable functions of $\gamma\delta$ T cells. *Cold Spring Harbor Symp Quant Biol* 1989; 54:31–44.
- 22 Vidovic D, Roglic M, McKune K et al. Qa-1 restricted recognition of foreign antigen by a γ/δ T cell hybridoma. *Nature* 1989; 340:646–50.
- 23 Havran WL, Chien YH, Allison JP. Recognition of self antigens by skin-derived T cells with invariant $\gamma\delta$ antigen receptors. *Science* 1991; 252:1430–2.
- 24 Born W, Happ MP, Dallas A et al. Recognition of heat shock proteins and $\gamma\delta$ cell function. *Immunol Today* 1990; 11:40–3.
- 25 Kabelitz D, Bender A, Schondelmaier S, Schoel B, Kaufmann SH. A large fraction of human peripheral blood $\gamma\delta$ ⁺ T cells is activated by *Mycobacterium tuberculosis* but not by its 65-kD heat shock protein. *J Exp Med* 1990; 171:667–79.
- 26 Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *New Eng J Med* 1989; 320:271–7.
- 27 Jeffrey PK, Wardlaw AJ, Nelson FC, Collins JV, Kay AB. Bronchial biopsies in asthma. An ultrastructural, quantitative study and correlation with hyperreactivity. *Am Rev Respir Dis* 1989; 140:1745–53.

- 28 Taylor KJ, Luksza AR. Peripheral blood eosinophil counts and bronchial responsiveness. *Thorax* 1987;42:452–6.
- 29 Sedgwick JB, Calhoun WJ, Gleich GJ et al. Immediate and late airway response of allergic rhinitis patients to segmental antigen challenge. Characterization of eosinophil and mast cell mediators. *Am Rev Respir Dis* 1991; 144:1274–81.
- 30 Griffin E, Hakansson L, Formgren H et al. Blood eosinophil number and activity in relation to lung function in patients with asthma and with eosinophilia. *J Allergy Clin Immunol* 1991; 87:548–57.
- 31 Walker C, Virchow JC Jr, Bruijnzeel PL, Blaser K. T cells and asthma. II. Regulation of the eosinophilia of asthma by T cell cytokines. *Int Arch Allergy Appl Immunol* 1991; 94:248–50.
- 32 Fujisawa T, Abu-Ghazaleh R, Kita H, Sanderson CJ, Gleich GJ. Regulatory effect of cytokines on eosinophil degranulation. *J Immunol* 1990; 144:642–6.
- 33 Tai PC, Sun L, Spry CJ. Effects of IL-5, granulocyte/macrophage colony-stimulating factor (GM-CSF) and IL-3 on the survival of human blood eosinophils in vitro. *Clin Exp Immunol* 1991; 85:312–6.
- 34 Broide DH, Paine MM, Firestein GS. Eosinophils express interleukin 5 and granulocyte macrophage-colony stimulating factor mRNA at sites of allergic inflammation in asthmatics. *J Clin Invest* 1992; 90:1414–24.
- 35 Dubucquoi S, Desreumaux P, Janin A et al. Interleukin 5 synthesis by eosinophils: association with granules and immunoglobulin-dependent secretion. *J Exp Med* 1994; 179:703–8.
- 36 Kay AB. T lymphocytes and their products in atopic allergy and asthma. *Int Arch Allergy Appl Immunol* 1991; 94:189–93.
- 37 Frew AJ, Corrigan CJ, Maestrelli P et al. T lymphocytes in allergen-induced late-phase reactions and asthma. *Int Arch Allergy Appl Immunol* 1989; 88:63–7.
- 38 Bennich H, Johansson SGO. Structure and function of human immunoglobulin E. *Adv Immunol* 1971; 13:1–55.
- 39 Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989; 7:145–73.
- 40 Geléa P, Brezinschek R, Lipsky PE, Oppenheimer-Marks N. Phenotypic characterization of CD4⁺/αβ TCR⁺ and γδ TCR⁺ T cells with a transendothelial migratory capacity. *J Immunol* 1994; 153:529–42.
- 41 Walker C, Kaegi MK, Braun P, Blaser K. Activated T cells and eosinophilia in bronchoalveolar lavages from subjects with asthma correlated with disease severity. *J Allergy Clin Immunol* 1991; 88:935–42.
- 42 Wen L, Roberts SJ, Viney JL et al. Immunoglobulin synthesis and generalized autoimmunity in mice congenitally deficient in αβ (+) T cells. *Nature* 1994; 369:654–8.
- 43 McMenamin C, Pimm C, McKersey M, Holt PG. Regulation of IgE responses to inhaled antigen in mice by antigen-specific γδ T cells. *Science* 1994; 265:1869–71.
- 44 Lundqvist C, Baranov V, Teglund S, Hammarström S, Hammarström ML. Cytokine profile and ultrastructure of intraepithelial γδ T cells in chronically inflamed human gingiva suggest a cytotoxic effector function. *J Immunol* 1994; 153:2302–12.
- 45 Qian D, Sperling AI, Lancki DW et al. The γ chain of the high-affinity receptor for IgE is a major functional subunit of the T-cell antigen receptor complex in γδ T lymphocytes. *Proc Natl Acad Sci* 1993; 90:11875–9.

Copyright of Clinical & Experimental Allergy is the property of Blackwell Publishing Limited and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.