

# IMBALANCE OF T-CELL SUBSETS IN IRANIAN ALLERGIC RHINITIS PATIENTS

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*Abstract*—Twenty-nine patients were studied to determine the number of CD4<sup>+</sup> T-cell subsets in Iranian allergic rhinitis patients. The result was compared with that of twenty-four nonatopic controls. The number of B and CD8<sup>+</sup> T cells and levels of specific IgE and IgG<sub>4</sub> against weed pollens were also studied. The number of lymphocyte subset, that is, CD4<sup>+</sup> CD29<sup>+</sup> and CD4<sup>+</sup> CD45R<sup>+</sup> cells were determined by double-labeling immunofluorescence assay. The results showed that the number of CD4<sup>+</sup> CD45R<sup>+</sup> cells is significantly higher in the patients (626 ± 251.4 cells per one microliter of peripheral blood) than in control subjects (479.4 ± 78.2 cell per one microliter) (*P* < 0.05). However, there was no statistical difference between the number of CD4<sup>+</sup> CD29<sup>+</sup> cells in the two groups. There were no correlation between the number of CD4<sup>+</sup> CD45R<sup>+</sup> cells in the peripheral blood of allergic patients and levels of total and specific IgE, but a direct correlation was found between the levels of specific IgE and specific IgG<sub>4</sub> in the sera of allergic patients (*r* = 0.55, *P* < 0.002). *Acta Medica Iranica* 33 (3&4): 69-73; 1995

*Key words:* Iranian allergic rhinitis; lymphocyte subsets; CD4<sup>+</sup> CD45R<sup>+</sup> cells; specific IgE; specific IgG<sub>4</sub>

## INTRODUCTION

Allergic rhinitis is one of the atopic diseases that IgE plays an important role in its pathogenesis. IgE responses are mainly regulated by T cells (1), through their helper and suppressor activities (2). Cytokines play a major role in these T-cell functions (2,3). Some investigators showed evidence that the number of suppressor T cells is abnormal in atopic patients (4) and allergen specific suppressor activity is induced during ragweed desensitization of atopic patients (5). However, there are conflicting reports about changes in CD4 to CD8 ratio in the peripheral blood of these individuals (4,6,7). In general, it seems that changes in the peripheral blood lymphocytes of atopic patients may not be evaluated by CD4/CD8 subset analysis and apparently more detailed

analysis of T-cell phenotypes might be required. In this study, we determined the number of CD4<sup>+</sup> CD45R<sup>+</sup> and CD4<sup>+</sup> CD29<sup>+</sup> peripheral blood T cells of Iranian allergic rhinitis patients. In addition, the number of B and CD8<sup>+</sup> T cells and levels of specific IgE and IgG<sub>4</sub> against common weed pollen allergens were studied.

## MATERIALS AND METHODS

### Subject

Twenty-nine patients (aged 22 to 32 years, median: 27) with allergic rhinitis were chosen on the basis of medical history and symptoms of rhinitis during the weed-pollen season (April through July) and positive skin prick test to weed pollen extracts. None of the twenty-four healthy age-matched controls (aged 23 to 30 years, median: 27) had medical history and symptoms of rhinitis during the year and all had negative skin prick test. To lessen age effect, the patients and controls selected were in the same age group. Furthermore, none of the patients had received immunotherapy. All sera were collected in the season that natural exposure to weed pollen allergens existed and was stored in -20°C until needed.

### Prick skin test

The allergenic extracts for this test were purchased from Dome/Hollister (U.S.A.). Histamine and glycerol were used as positive and negative controls, respectively. Test was performed according to the standard procedure (13).

### Antibody measurement

Total IgE level in the serum of each individual was determined using a commercial ELISA kit (Behring Werke AG, W. Germany), and according to the manufacturer's recommendations.

Specific IgE against common weed pollens in the area (Lambs quarter and Russian thistle-W<sub>10</sub> + W<sub>11</sub>) was determined using a commercial ALASTAT kit (DPC, California), as recommended by the manufacturer. Specific IgG<sub>4</sub> against Lambs quarter and Russian thistle

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was evaluated by an enzymeimmunoassay procedure. Allergen disks (Pharmacia Diagnostics, Sweden) were used as source of antigen. Before use, disks were saturated by Bovin serum albumin (Behring Werk AG) to block any remaining protein-binding sites on them. After washing, 100 microliters of undiluted serum was added to the disk and incubated for 120 minutes at room temperature.

In blank wells, washing buffer was added and after washing for three times, 100 microliters of a 1/500 diluted Biotin conjugated anti-IgG<sub>4</sub> (Clone HP-6025, Sigma Chemical Co.) were added and incubated for 90 minutes at room temperature. Following washing for three times, 100 microliters of a 1/300 diluted avidin peroxidase (Sigma Chemical Co.) were added and incubated for 30 minutes at room temperature. Again, after washing for three more times, 100 microliters of substrate (34 mg O-phenylenediamine and 50 microliters of hydrogen peroxide in 100 milliliters of 0.1 molar citrate-phosphate buffer, PH=5.0) were added and when a pale color developed in the negative control, the reaction was stopped by adding 25 microliters of 3 molar sulfuric acid to each disk. Then the disk was removed from reaction, and the color of the soluble phase of the reaction was evaluated in an ELISA reader, set at 492 nanometre. Relative optical density (ROD) was calculated as follows:

$$ROD = \frac{OD (test) - OD (blank)}{OD (positive control) - OD (bank)}$$

where, positive control refers to pooled sera from 14 rhinitis patients who had been under prolonged immunotherapy with W<sub>10</sub> + W<sub>11</sub>. NET buffer was used in all washing steps and dilutions. This buffer contained NP-40 as a detergent in Tris buffer.

**Cell staining and flow cytometry**

Cells in the whole blood were stained according to the manufacturer's recommendations (Coulter Electronics). B lymphocytes and T-cell subsets were enumerated by double labeling immunofluorescence, using rhodamine and FITC conjugated monoclonal antibodies (T<sub>11</sub>/B<sub>4</sub>, T<sub>4</sub>/B<sub>4</sub>, T<sub>4</sub>/2H<sub>4</sub> and T<sub>4</sub>/4B<sub>4</sub>) (Coulter Electronics, Krefeld, FRG) (2H4 and 4B4 are mAbs that determine CD45R and CD29, respectively). Ten-thousand events were counted using a profile II (Coulter Electronics), equipped with Argon laser source. Data were expressed as absolute number of lymphocytes and their subsets in each microliter of peripheral blood.

**Statistical analysis**

Differences between the study groups were tested

using the non-parametric Mann-Whitney U-test. The associations between total IgE, specific IgE and absolute number of lymphocyte subsets and the correlation between specific IgE and specific IgG<sub>4</sub> were tested by nonparametric Sperman-rank correlation (14).

**RESULTS**

Absolute numbers of B-cell and T-cell subsets (CD8<sup>+</sup> and CD4<sup>+</sup> CD29<sup>+</sup>) did not differ significantly when the allergic rhinitis patients were compared with control group (Figs. 1,2, and 3). However, patients showed significantly higher number of CD4<sup>+</sup> CD45R<sup>+</sup> lymphocytes than that of control group (P < 0.05) (Fig. 4).

As shown in Figures 5 and 6, there were no significant correlartions between the amount of specific IgE and CD4<sup>+</sup> CD45R<sup>+</sup> cells (r=0.13) and the amount of total IgE and CD4<sup>+</sup> CD45R<sup>+</sup> cells (r=0.04). We found a significant correlation (r=0.55, P < 0.002) between specific IgE and IgG<sub>4</sub> production in the allergic patients (Fig. 7).

**DISCUSSION**

We did not find any changes in the absolute numbers of CD4<sup>+</sup> and CD8<sup>+</sup> -T-cells in the peripheral blood of atopic patients, compared to those of the normal group, which was in accordance with previous findings (6,19). This might be related to the nature of allergic rhinitis, which is a localised (and not a systemic) IgE-dependent hypersensitivity.

The current study showed that the number of CD4<sup>+</sup>

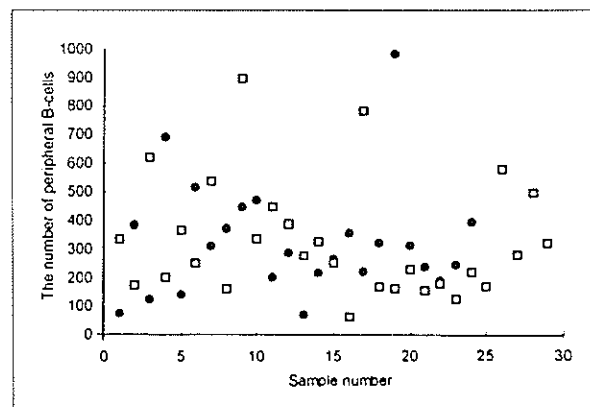


Fig. 1. The number of B cells in the peripheral blood of rhinitis patients (314±169) and normal subjects (325±196).

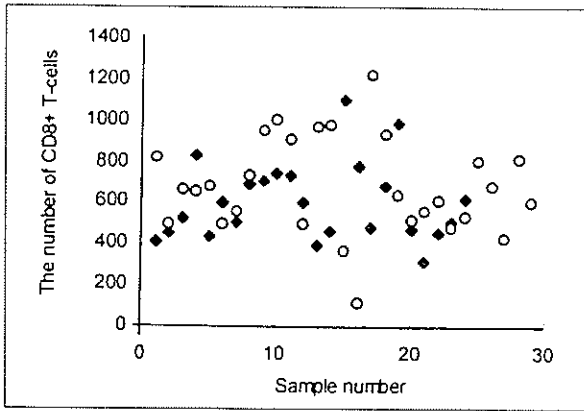


Fig. 2. The number of peripheral blood CD8<sup>+</sup> T cells in the rhinitis patients (681±230) and normal subjects (605±187).

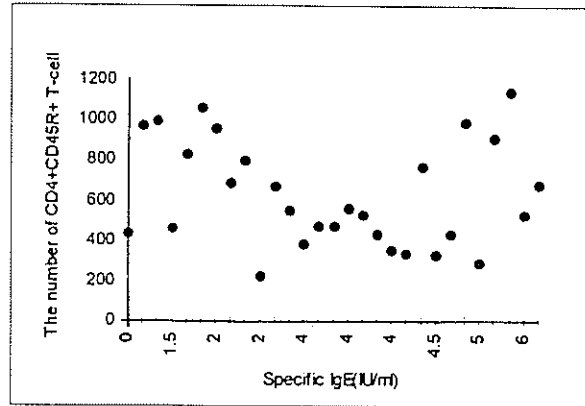


Fig. 5. Weed pollen (W10+W11) specific IgE (determined by ALASTAT) were not correlated with the number of peripheral blood CD4<sup>+</sup> CD45R<sup>+</sup> T cells in the rhinitis patients (n=29, r=0.1).

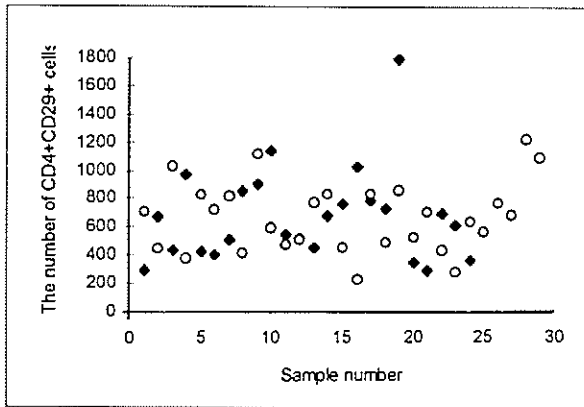


Fig. 3. The number of peripheral blood CD4<sup>+</sup> CD29<sup>+</sup> cells in the rhinitis patients (667±246) and normal subjects (673±325).

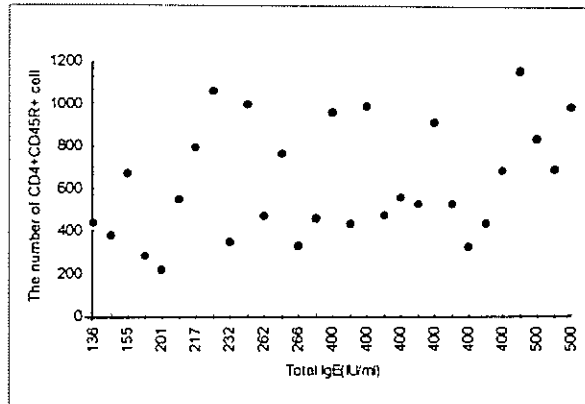


Fig. 6. Total IgE were not correlated with the number of peripheral blood CD4<sup>+</sup> CD45R<sup>+</sup> T cells in the rhinitis patients (n=29, r=0.1).

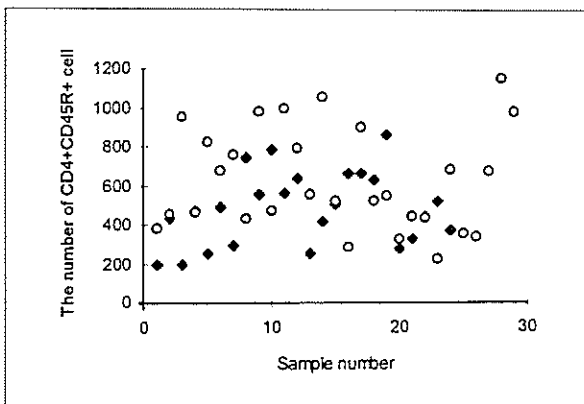


Fig. 4. The number of peripheral blood CD4<sup>+</sup> CD45R<sup>+</sup> cells in the rhinitis patients (626±251) and normal subjects (448±178) (p < 0.05).

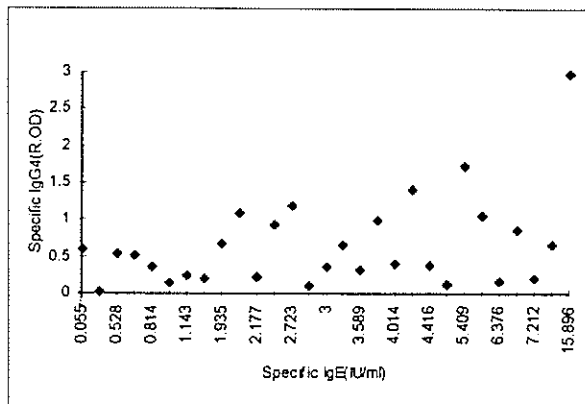


Fig. 7. Weed pollen (W10+W11) specific IgE were correlated with (W10+W11) specific IgG<sub>4</sub> in the sera of rhinitis patients (n=29, r=0.55, p < 0.002).

CD45R<sup>+</sup>-T lymphocytes had significantly increased in the peripheral blood of Iranian allergic rhinitis patients, whereas the number of CD4<sup>+</sup> CD29<sup>+</sup> cells had not. Recent studies indicate that CD4<sup>+</sup> CD45R<sup>+</sup> and CD4<sup>+</sup> CD29<sup>+</sup> lymphocytes represent maturational stages within the same Lineage (15,16).

Stimulation of CD4<sup>+</sup> CD45R<sup>+</sup>A cells by mitogen led to increased density of CD29 and loss of the CD45RA marker. Furthermore, the mature CD4<sup>+</sup>CD45R<sup>+</sup> cells responded poorly (17). Now, it is clearly evident that CD4<sup>+</sup> CD45R<sup>+</sup> and CD4<sup>+</sup> CD29<sup>+</sup> represent native and memory cells, respectively. Interestingly, a poor response to recall antigens was shown in atopic patients (18). Other investigators have shown that the proportion of CD<sup>+</sup> CD45R<sup>+</sup> cells decline with age but it is less so in the atopic patients (19).

Recently, it was shown that as CD45R<sup>LO</sup>-T cells develop into a cell line and secrete IL-4, they become CD45R<sup>hi</sup>. In contrast, CD45R<sup>LO</sup> cells develop into a cell line that secretes IL-2 and maintains their CD45R<sup>LO</sup> phenotype (20). This suggests that both Th<sub>1</sub> and Th<sub>2</sub> cells arise from CD45R<sup>LO</sup> cells; Th<sub>2</sub> cells become CD45R<sup>hi</sup> during prolonged in vitro culture, whereas Th<sub>1</sub> cells do not (20). Thus with respect to the role of IL-4 as a IgE-switching factor, increased number of CD4<sup>+</sup> CD45R<sup>+</sup> cells in the patient group was an expected phenomena compared with that of the normal group. Since, we did not find any correlation between the number of CD4<sup>+</sup> CD45R<sup>+</sup> cells and the levels of total or specific IgE in allergic patients (Figs. 5 and 6), it would be informative if the cytokine profile of CD4<sup>+</sup> CD45R<sup>+</sup> cells are further studied.

The role of IL-4 as a IgE-switching factor has been established (21). In mice and humans, two distinct patterns of cytokine production have been defined among CD4<sup>+</sup> T cells (22,23). Th<sub>1</sub> cells produce IL-2, IFN-γ and TNF-β, whereas Th<sub>2</sub> cells secrete IL-4, IL-5, IL-10, and IL-13 (28). Most allergen or helminth-antigen specific human CD4<sup>+</sup> T cells exhibit a Th<sub>2</sub> phenotype. Allergen specific Th<sub>2</sub> cells seem to play a crucial role in atopy. These cells induce IgE production via IL-4 and IL-13 and favour the differentiation and activation of eosinophils via IL-5.

It was shown that allergen reactive Th<sub>2</sub> cells have tendency to occur at a higher frequency with increasing production of Dematophagoides pteronyssinus-specific IgE (24). Therefore, there is a relation between the number of specific Th<sub>2</sub> cells and allergen specific IgE production. In this study we were unable to show any correlation between the number of CD4<sup>+</sup> CD45R<sup>+</sup> cells and levels of specific or total IgE in the sera of the patients. Probably, this was because we did not study allergen-specific CD4<sup>+</sup> CD45R<sup>+</sup> cells.

Another important point in this study was the correlation between the levels of specific IgE and specific IgE<sub>4</sub> against W<sub>10</sub> + W<sub>11</sub> (r=0.55, P < 0.002). Marsh et al (25) showed that in the sera of allergic patients with specific IgE, significant amount of specific IgG can be detected, and there is also good correlation between specific IgE and IgG in the sera of untreated atopic individuals. Most of these IgG antibodies belong to IgG<sub>4</sub> subclass (26) and the total IgG<sub>4</sub> rises in these patients (27).

Correlation between specific IgG<sub>4</sub> and IgE can be explained by the important role of IL-4 in switching to IgE and IgG<sub>4</sub> (28).

## REFERENCES

1. Ishizaka K, Ishizaka T. In: Paul W, ed. *Fundamental Immunology* Second Edition New York: Raven press; 1989: 867-888.
2. Romagnani S. Regulation of human IgE synthesis. *Immunol Today* 11: 316-321; 1990.
3. Paul W, Seder RA. Lymphocyte responses and cytokines. *Cell* 76: 241-251; 1994.
4. Leung D, Rhodes A, Geha R. Enumeration of T-cell subsets in atopic dermatitis using monoclonal antibodies. *J Allergy Clin Immunol.* 67: 450-455; 1981.
5. Lesourd B, Paupe J, Thiollet M, Moutias R, Sainte-Laudy J, Scheinmann P. Hymenopteran venom Immunotherapy: I. Induction of T cell mediated immunity by honey bee venom immunotherapy: Relationships with specific antibody responses. *J Allergy Clin Immunol.* 83: 563-571; 1989.
6. Matloff S, Kiselis I, Rocklin R. Reduced production of histamine-induced suppressor factor (HSF) by atopic mononuclear cells and decreased prostaglandin E<sub>2</sub> output by HSF-stimulated atopic monocytes. *J Allergy Clin Immunol.* 72: 359-364; 1983.
7. Kus J, Tse K, Vedal S, Chan YM. Lymphocyte subpopulations in patients with allergic and non-allergic asthma. *Clin Allergy* 523: 529; 1985.
8. Barbee R, Halonen M, Lebowitz M, Burrows B. Distribution of IgE in a community population sample: correlations with age, sex and allergen skin test reactivity. *J Allergy clin Immunol.* 86: 106-111; 1981.
9. Warren W, Holford Strevens V, Wong C, Manfreda J. The relationship between smoking and total immunoglobulin E levels. *J Allergy clin Immunol.* 69: 370-375; 1982.
10. Lichtenstein LM, Ishizaka K, Norman PS, Kagey-Sobotka A, Hill BM. IgE antibody measurements in ragweed hay fever: Relationship to clinical severity and the results of immunotherapy. *J clin Invest.* 52: 472-482; 1973.
11. Westermann J, Pabst R. Lymphocyte subsets in the blood: a diagnostic window on the lymphoid system. *Immunol Today* 11: 406-410; 1990.
12. Canonica G, Mingari M, Melioli G, Colombatti M, Moretta L. Imbalances of T-cell subpopulations in patients with atopic

diseases and effect of specific immunotherapy. *J Immunol* **123**: 2669-2672; 1979.

13. Bosquet J, Michel F. In vivo methods for study of allergy. In: Middleton E Jr, Reed CE, Ellis E, Adkinson NF, Yungineer JW, Busse WW, eds. *Allergy principles and practice*. St. Louis: Mosby Year Book; 1993: 573-594.

14. Conover WJ. *Practical nonparametric statistics*. Second Edition. New York: John Wiley and Sons; 1980: 200-240.

15. Akbar A, Terry L, Timms A, Beverley P, Janossy G. Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. *J Immunol* **140**: 2171-2178; 1988.

16. Sanders M, Makgoba M, Shaw S. Human native and memory T cells, reinterpretation of helper-inducer and suppressor inducer subsets. *Immunol Today* **9**: 195; 1988.

17. Beverley P. Human T-cell subsets. *Immunol Lett* **14**: 263; 1987.

18. Megeady S, Buckley R. Depression of cell mediated immunity in atopic eczema. *J Allergy Clin Immunol* **56**: 393-406; 1975.

19. Schauer U, Jung T, Heymanns J, Rieger CHL. Imbalance of CD4<sup>+</sup> CD45R<sup>+</sup> and CD4<sup>+</sup> CD29<sup>+</sup> T-helper cell subsets in patients with atopic diseases. *Clin Exp Immunol* **83**: 25-29; 1991.

20. Lee W, Vitetta E. Changes in expression of CD45R during the development of Th<sub>1</sub> and Th<sub>2</sub> cell lines. *Eur J Immunol* **22**: 1455-1459; 1992.

21. Coffman R, Ohara J, Bond M, Carty J, Zlotnik A, Paul W. B-cell stimulatory factor-1 enhances the IgE response of lipopolysaccharide activated B cells. *J Immunol* **136**: 4538-4541; 1986.

22. Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RI. Two types of murine helper T-cell clone. I: Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* **136**: 2348-2357; 1986.

23. Romagnani S. Human Th<sub>1</sub> and Th<sub>2</sub> subsets, doubt no more. *Immunol Today* **12**: 256-257; 1991.

24. Wierenga E, Snoek M, de Groot C, Chretien I, Bos J, Jansen H, Kapsenberg M. Evidence for compartmentalization of functional subsets of CD4<sup>+</sup> T lymphocytes in atopic patients. *J Immunol* **144**: 4651; 1990.

25. Marsh D, Hu S, Roebber M, Ehrlich-Kautzky E, Freidhoff L, Meyers D, et al. HLA-DW<sub>2</sub>: A genetic marker for human immune response to short ragweed pollen allergen Ra5. I: Response resulting primarily from natural antigenic exposure. *J Exp Med* **155**: 1439-1451; 1982.

26. Devey M, Wilson D, Wheeler A. The IgG subclasses of antibodies to grass pollen allergens produced in hay fever patients during hyposensitization. *Clin Allergy* **6**: 227; 1976.

27. Merrett J, Barnetson R, Burr M, Merrett T. Total and specific IgG<sub>4</sub> antibody levels in atopic eczema. *Clin Exp Immunol* **56**: 654-652; 1984.

28. Gascan H, Gauchat JF, Aversa G, Van Vlasselaer P, Devries JE. Anti CD40 monoclonal Abs or CD4<sup>+</sup> T clones and IL-4 induce IgG<sub>4</sub> and IgE switching in purified human B cells via different signaling pathways. *J Immunol* **147**: 8-13; 1991.

