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SPECIAL ARTICLE

Immunological Defects in Allergic Asthmatic Children and Working Mechanisms of Hyposensitization--A Review

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SUMMARY **Patients with allergic diseases are characterized by the presence of elevated serum IgE and specific IgE antibodies against a variety of environmental allergens, especially house dust, mites and molds in Taiwan. A series of studies on allergic asthmatic children have been conducted to explore the non-immunological and immunological causes for their augmented production of IgE antibody and to explore the working mechanisms of hyposensitization. The results showed that the patients had multiple defects in their defense mechanisms, including hyper-permeability of mucosae, hyperreactivity of target organs, defective phagocyte functions, and deficient helper and suppressor T-cell functions. Hyposensitization was able to partially correct the immunological aberrations. More over, a newly found lymphokine termed "T-cell growth factor" or "interleukin 2" may be used not only as an indicator for the initiation and termination of hyposensitization, but may also provide a promising tool for the treatment of allergic diseases in the future because of its capability of enhancing and expanding allergen-specific suppressor T cells.**

The human defense mechanism against environmental invaders is composed chiefly of two major parts. One is physicochemical barriers such as skin, mucosae, mucus, digestive enzymes and pH of the respiratory and gastrointestinal tracts; the other is immune surveillance which includes non-specific and specific immune reactions (both local and systemic). The non-specific immune reactions include the phagocytosis of polymorphonuclear and mononuclear leukocytes, natural killer cells, K cells, lymphokine-activated killer (LAK) cells, and complement components and their activated products. Specific immune reactions include the establishment and expansion of antigen-specific T cells with helper, suppressor and cytotoxic functions and B cells, producing and secreting antibodies against specific antigens. In fact, all of these immunological functions are only artificially dissected for convenience of discussion; *in vivo* they cooperate and act in concordance to effectively protect human beings from injury caused by foreign substances such as microorganisms.

The specific immune reaction is initiated with phagocytosis of antigens by monocytes/macrophages. The process of phagocytosis triggers a series of intracellular biochemical changes and the monocytes are activated. The activated monocytes not only present the antigen they processed to helper T cells (which share the same Ia-like molecular product of human HLA-DR), but they also secrete a number of immunologically active molecules such as monokines (e.g., interleukin 1, IL-1)[1] which are capable of regulating the immunobiological functions of other immunocompetent cells. After activation by antigen and IL-1, the helper T-cells start to proliferate and transmit antigenic information to suppressor T cells, cytotoxic T cells and B cells via both cellular interaction or via effects soluble molecules, such as lymphokines (e.g., interleukin 2 or interleukin 3, see below). A complex network of specific immune responses is thus finally established.[2]

Elevation of serum IgE antibody is one of the characteristics of patients with allergic diseases.[3-5] Although several non-immunological factors are capable of triggering the occurrence of allergic diseases such as bronchial asthma, hay fever, atopic eczema and urticaria, the specific antigen-IgE antibody reaction still plays an important role in the pathogenesis of these diseases.

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The bridging of two cell-bound IgE molecules by a specific allergen triggers the basophils and mast cells to release mediators such as histamine and leukotrienes (SRS-A) which in turn act on the specific target organ and result in various allergic symptoms.[6]

An abnormal immune response may result from a defect at any stage, beginning from the entrance of a foreign substance into the body past a physical barrier through the recognition of the antigen by various immunocompetent cells. However, as it is ethically impossible in human beings to study *in vivo* immunoregulation by using irradiation, thymectomy and immunosuppressive agents, etc., as is done in animal models, we take advantage of hyposensitization (desensitization or immunotherapy) to study the *in vivo* immunoregulatory function in allergic patients.

Since Noon's report in 1911,[7] hyposensitization has been widely accepted as a specific treatment for allergic diseases and it has been shown repeatedly to be clinically effective, especially for respiratory allergies and hymenoptera hypersensitivity.[8,9] Hyposensitization is a procedure of long-term, regular administration to sensitive patients of steadily increasing doses of allergen; it results finally in the amelioration of allergic symptoms. For more than half a century, house dust has been implicated as one of the most important perennial allergens world-wide,[10-12] especially in Taiwan.[13,14] By using house dust as allergen, a series of studies have been carried out on asthmatic children to explore the immunological and non-immunological defects in allergic individuals and the working mechanisms of hyposensitization.

The study population consisted of asthmatic children who visited the Allergy Clinic of the Department of Pediatrics at the National Taiwan University Hospital. A diagnosis of bronchial asthma was made based on the history of recurrent, paroxysmal attacks of reversible, obstructive airway disease which resolved spontaneously or after treatment with bronchodilators. The allergic nature of asthma was diagnosed by the presence of a personal or family allergy history, eosinophilia, increased total serum IgE and positive skin test and a radioallergosorbent test (RAST) to house dust. Hyposensitization was started with weekly injections and the allergen dose was increased as rapidly as possible until a maximal tolerated dose was reached (usually within six months). The dose was then maintained and administered at four-week intervals for two-three years. The house dust was prepared in our laboratory and could elicit positive skin tests in 75% of the asthmatic children, could be used to detect circulating house-dust-specific IgE antibody in 72.1% of patients and could trigger asthmatic attacks in 19.1% of patients during the course of hyposensitization.[14]

Excessive production of antibodies including IgE may result from continuous stimulation of the immune system by antigen and by the abnormal response of lymphoid organs to such stimulation. This communication summarizes our recent studies on the possible mechanisms accounting for augmented production of IgE antibody in allergic patients and on the working mechanisms of hyposensitization.

Salvaggio *et al*[15,16] reported that the immune response to an intranasally applied allergen was greatly enhanced in atopic patients when compared to that of normal individuals, indicating mucosa hyperpermeability in the former. Levine *et al*[17] proposed that repeated exposures to minute amounts of antigen predisposed atopic individuals to develop an immune response in which IgE antibody is predominant. Other studies have indicated that allergen and/or immune complexes persist in the circulating blood of allergic patients.[18-20] Therefore, it may be proposed that augmented IgE production results from the continuous stimulation of atopic individuals by minute amounts of allergen circulating in the blood because of a defect in the mucosal barrier function. The bronchial provocation test (BPT) was therefore performed in asthmatics to study the barrier function of their respiratory mucosa.[13] The patients were instructed to inhale continuously increasing amounts of house dust until an asthmatic attack occurred (positive BPT) or until the maximal dose of allergen was reached with no attack (negative BPT). Blood was then drawn from which the complement levels and the total eosinophil counts were determined. The results showed that ten out of the 20 skin test-positive asthmatic children gave a positive BPT and that among them, half developed eosinophilia and 3 had decreased complement levels. Seven out of the 10 BPT-negative patients also developed eosinophilia. However, none of the healthy subjects showed such changes. Since house dust has been shown to be capable of activating complement *in vitro*,[21,22] and since eosinophilia is just a non-specific reflection of antigen challenge *in vivo*,[23] it was concluded that the asthmatic patients, in whom the bronchial tree is the target organ, do have defects in the barrier function of their respiratory mucosa, i.e. hyperpermeability. This defect may permit the environmental allergens to enter the body more easily and to stimulate the immune system continuously.

The phagocytic function of both polymorphonuclear and mononuclear leukocytes greatly affects the fate of antigens after they enter the circulation. Frank *et al*[24] reported defective Fc

receptor function of the reticuloendothelial system in systemic lupus erythematosus, a disease characterized by the occurrence of hypergammaglobulinemia and multiple autoantibodies, and they proposed that failure of the phagocytes to efficiently eliminate the circulating antigen might contribute to the overproduction of antibodies and immunoglobulins due to continuous stimulation of the lymphoid tissue by antigen. As the phagocyte functions (e.g., phagocytosis, bacterial killing, chemotaxis and adherence) correlate closely with the Fc and complement receptors on their surfaces,[25,26] the numbers of phagocytes with Fc and complement receptors were enumerated.[27] The results showed that the numbers of Fc and complement receptor-bearing neutrophils and monocytes in asthmatics were much lower than those of normals, and that the difference became bigger after allergen challenge. Further study showed that in addition to these intrinsic defects, many drugs commonly used in the treatment of allergic diseases exerted adverse effects, even in the therapeutic range, on phagocyte functions, including the expression of Fc and complement receptors.[28] Thus, the hyperpermeability of the mucosa and the defective expression of Fc and complement receptors on the phagocytes may have an additive adverse effect on the defense mechanism of the human body in terms of antigen clearance and they may favor the occurrence of IgE-mediated allergic diseases.

The production of IgE is a highly T cell-dependent process,[29] and a delicate balance between helper T (Th) and suppressor T (Ts) cells is absolutely required for maintaining a normal IgE response to antigenic stimulation. In recent years, both the numbers and functions of T cell subsets in allergic patients have been studied extensively.

First of all, the mean percentages and absolute numbers of T cells were enumerated by E-rosetting technique. A lymphocyte which binds three or more sheep RBC is defined as a T cell (E-rosette). Total T cells represent the total number and/or percentage of lymphocytes which form E-rosettes after being incubated with neuraminidase-treated SRBC at 4°C for at least one hour or over-night,[30] and active T cells represent the portion of total T cells which are able to form E-rosettes within 5 minutes after being incubated with neuraminidase-untreated SRBC at 37°C.[31] The results showed that there was no difference in the mean percentages of total T and active T cells in asthmatics as compared with healthy children. Moreover, because of the frequent relative lymphocytosis in asthmatics, their absolute numbers of total and active T cells were higher than those in normal individuals.[3] This result was in contrast to that reported by Gupta *et al*[32] but was in agreement with that reported by McGeady *et al.*[33]

Since a normal number of T cells does not necessarily mean that T-cell function is also normal, the regulatory function of T cells in asthmatic children was also studied, utilizing the model of *in vitro* IgE biosynthesis. This study was conducted by the co-cultivation method of Waldmann *et al.*[34] Mononuclear cells (MNC) from asthmatic children with elevated serum IgE and mononuclear cells from normal individuals were cultured alone or were co-cultivated. The suppressor T-cell function of the patient was considered to be deficient if the IgE concentration in the co-cultured supernatant was less than 50% of the sum of the concentrations for single cultures of normals and of patients. Results showed that the suppressor T-cell function was deficient in at least some of the asthmatics.[4]

As in rodent models, IgE production can be enhanced by procedures which eliminate T cells. These procedures include irradiation, thymectomy, splenectomy, administration of various immunosuppressive agents and administration of anti-thymocyte serum.[29] An attempt was made to study the effects of some drugs with immunoregulatory capability on the *in vitro* IgE biosynthesis.[35] These included cyclophosphamide (CY, 200 μg/ml), a suppressor T cell inhibitor; concanavalin A (Con A, 10 μg/ml), a suppressor T cell activator; and levamisole (1 μg/ml), a non-specific immune stimulator. The results showed that CY was able to enhance IgE production significantly in 9 out of 21 asthmatic children but in none of the normals; and neither Con A nor levamisole affected the IgE synthesis in either patients or normals. The fact that lymphocytes from allergic children were still able to produce more IgE after treatment with CY was comparable to the result observed in high IgE responder mice.[36] As similar treatment of lymphocytes from normals did not show the same effect, it may be speculated that heightened IgE production by CY-treated lymphocytes from allergic individuals was due to a weaker and more vulnerable IgE damping mechanism of suppressor T cells rather than to damping insensitivity of the IgE B cells as postulated by Chiorazzi *et al.*[36] Con A has been shown to be able to generate suppressor T cells for pokeweed mitogen-induced polyclonal immunoglobulin production,[37] but in this study Con A at a concentration of 10 μg/ml failed to generate IgE isotype-specific suppressor T cells. Another study found that, after treatment with 20 μg/ml or more of Con A for 18 hours, T cells from both normals and allergic subjects were able to suppress IgG, but not IgE production of allogeneic lymphocytes.[38] The results suggest that T-cell regulation of IgE production is different from that of IgG production.

The possibility that the T cells of allergic patients act quite differently from those of normals is further supported by a study of the autologous mixed lymphocyte reaction (AMLR). The *in*

vitro proliferation of T cells in response to stimulation by autologous non-T cells has been called the AMLR. The cells responding in this reaction show immunological memory or specificities and exert a variety of effector functions including cytotoxic, helper and suppressor effects.[39] Our results showed that although there was no difference in the AMLR stimulation index, whether B cells, monocytes or a combination of B cells and monocytes were used as stimulators, the autoreactive T cells from normals were able to suppress *in vitro* IgE biosynthesis and the blastogenic response to PHA of fresh autologous MNC. On the other hand, those from allergic patients enhanced rather than suppressed both of these immunological phenomena.[40] Thus, the apparent difference in the immunoregulatory properties of T cells responding in AMLR between allergic patients and normals may be used to partly explain the augmented IgE production in atopic subjects.

In addition to intrinsic defects, suppressor T-cell deficiency that is secondary to increased adherent suppressor cell activity has been reported in patients with systemic lupus erythematosus,[41] tuberculosis,[42] chronic fungal infections[43] and malignancy.[44,45] This possibility was examined here in allergic patients.[46] We found that depletion of monocytes resulted in decreased IgE production by patient, but not by normal MNC. In recombination studies, addition of normal monocytes resulted in suppressed production of IgE by patient lymphocytes; on the other hand, addition of patient monocytes was able to enhance the IgE production by normal lymphocytes. Taken together, these data suggest that increased adherent suppressor cell activity may contribute to the suppressor T-cell deficiency in allergic patients.

As stated previously, most investigations reported normal total T and active T cells in allergic patients,[33,47,48] but the normal count could not rule out the possibility of quantitative or qualitative deficiency of a T-cell subpopulation (e.g., suppressor T cells) in these subjects. At the same time the study of human T-cell subsets (both numbers and functions) was hampered largely by the lack of surface markers like the Ly antigens in the murine system.[49] In 1977, Moretta *et al*[50] first demonstrated that T cells expressing receptors for Fc-IgM (T_μ cells) comprised the largest proportion of T cells (up to 60%) and acted as helpers for the proliferation and differentiation of B cells into immunoglobulin producing and secreting plasma cells. Of the remaining T cells, those expressing receptors for Fc-IgG (T_γ cells) comprised a smaller proportion of the T cells (up to 15%) and acted as suppressors after interaction with IgG immune complexes.

The distributions of T_μ and T_γ cells were studied in 57 asthmatic children without medication and in 23 normal healthy children.[51] There was no difference in the mean percentages of both T_μ and T_γ cells between the two groups. However, after stimulation *in vitro* with specific allergens, both T_μ and T_γ cells increased in the patients and only T_γ cells increased in the normals. The results were comparable to those reported by Ong *et al.*[52] It is therefore speculated that an enhanced T_γ cell population, after allergen challenge, may explain the normal IgE response in healthy individuals after natural allergen exposure.

By utilizing the hybridoma technique of Kohler and Milstein,[53] Reinherz *et al*[54] were able to develop a series of monoclonal antibodies against human T-cell differentiation antigens called the "OKT system" These antigens are comparable to the murine Ly antigens.[49] T cells carrying the OKT4 antigen possess the helper/inducer function and those carrying the OKT8 antigen possess the cytotoxic/suppressor function. This break-through makes the study of human T-cell subsets possible and contributes markedly to the understanding of the pathogenesis of immunologically mediated diseases including allergy.

T-cell subpopulations defined by OKT monoclonal antibodies were studied by the indirect immunofluorescent technique in 47 asthmatic children with or without atopic eczema and the results were compared to those for 26 normal subjects.[55] The distributions of T-cell subsets were studied in both MNC and purified T-cell populations. The latter were prepared by E rosetting followed by density gradient centrifugation. The mean percentage of OKT4 cells in the purified T-cell population of asthmatic children was much lower than that in normals. However, this difference disappeared after allergen-challenge, perhaps due to a slight but insignificant increase in OKT4 cells in the patients. A difference in OKT8 cells could not be found. There was no difference in the distribution of OKT4 and OKT8 cells in the MNC populations of the patients and the normals, but the OKT8 cells of both groups increased significantly after allergen challenge. The most interesting finding was that patients with atopic eczema, whether it was quiescent or still active at the time of study, had lower OKT4 cells than those without eczema. OKT4 cells in patients were lower in number only in the purified T cell population, not in the MNC population. This result might suggest that OKT4 cells in eczematous patients have a lower affinity for SRBC and that this abnormal function results in secondary suppressor T-cell deficiency due to lack of maturation induction and amplification by normal OKT4 cells.[54] Alternatively, a lack of suppressor T-cells within the OKT4 cell population may account for the decreased OKT4 cells in asthmatic children with atopic eczema.[56-58] These results contrast with those of Leung *et al*[59] who reported decreased OKT8 cells, rather than OKT4 cells in eczematous children. However, in a more recent study

of cell-mediated lympholysis, these authors reported that the function of both OKT8 cells and OKT4 cells was impaired in eczematous children.[60]

Hyposensitization has been accepted as a specific treatment for allergic diseases for mor than 70 years.[7] Several mechanisms have been postulated to account for its clinical effectiveness. These include: (1) increased allergen-specific IgG antibody which competes with cell-bound IgE for circulating allergen,[61,62] (2) decreased total serum IgE and blunted seasonal rises of allergen-specific IgE antibody,[63,64] (3) diminished mediator release from basophils and mast cells after allergen exposure,[62,65,66] (4) decreased lymphoproliferative responses and decreased production of lymphokines to allergen challenge,[67,68] (5) restoration of balance between helper and suppressor T cells[69] and (6) generation of antigen-specific suppressor cells.[70] The exact nature of the working mechanism of hyposensitization has still not been clearly delineated. Furthermore, the majority of the investigations have dealt with seasonal pollen allergens,[8,9] even though house dust has been recognized as one of the most important allergens the world over[10-14] and many patients have undergone hyposensitization treatment with it. As house dust is the most important allergen in Taiwan, especially in children,[13,14] our patients were treated with house dust, and immunological changes were followed serially, in the hope of delineating more clearly the working mechanisms of hyposensitization and providing a more solid rationale for its use in the treatment of allergic diseases.

After hyposensitization for one year, there was no change in the total serum IgE and house dust-specific IgE and IgG antibodies.[71] These results contrasted with those of most other studies since they often reported increased IgG antibody and decreased IgE antibody (especially seasonal changes), although the titers of the latter still remained more than high enough to sensitize mediator-containing cells.[63,64,68,72] The most important reason for this discrepancy may be the different antigens chosen for study. The house dust used in our studies is a perennial allergen and the dose entering the body through natural exposure is probably much larger than that administered during hyposensitization treatment.

One unexpected but very important result in our work concerned the changes of specific antibodies after immunotherapy. The specific IgG antibody, and thus the specific IgG/IgE antibody ratio, decreased in patients who frequently developed immediate type asthmatic reactions after allergen injections and who required reduction of the dose administered. This may be the first report which provides a possible explanation for the anaphylactic reactions which occur after long-term allergen administration.

Hyposensitization did not increase the Fc and complement receptor-bearing polymorphonuclear leukocytes but it enhanced their resistance to the adverse effect of allergen stimulation, i.e., their capability of maintaining normal receptor function after antigen challenge. Such a change may be of help in the clearance of circulating antigen.

The lymphoproliferative response to house dust was decreased after hyposensitization, while there was no change in the PHA-induced proliferation during the course of treatment. This result gives some indication of the specific effect of house-dust hyposensitization on house dust-induced *in vitro* lymphocyte reactivity and it agrees with the results of other reports in which ragweed,[67,68,73,74] grass,[72] alternaria,[75] and house dust[76] were used as allergens for treatment. The decreased house dust-induced lymphocyte blastogenesis may be explained by: (1) the blunted T_{μ} and T_{γ} proliferative responses to house dust challenge after hyposensitization,[71] (2) the decreased proliferation of OKT4 helper T cells in response to house dust stimulation,[77] (3) the enhanced house dust-induced proliferation of OKT8 suppressor T cells,[77] and (4) the generation of house dust-specific suppressor cells.[78] It is interesting to note that normal subjects also possess dust-specific suppressor cell function of a magnitude comparable to that of hyposensitized patients. Generation of antigen-specific suppressor cell activity had also been reported by Rocklin *et al*,[70] but the method of study was quite different and normal individuals were not examined at the same time.

In 1976, Morgan *et al*[79] first showed that T cells could be selectively grown and maintained for more than nine months when unfractionated normal human bone marrow cells were cultured with crude PHA-stimulated lymphocyte conditioned media. The factor present in the conditioned media and responsible for the continuous growth of T cells has now been well characterized and named T-cell growth factor (TCGF) or interleukin 2 (IL-2). IL-2 is one of the most important lymphokines secreted mainly by helper T cells after stimulation with mitogens or antigens.[80-82] Human IL-2 is a glycoprotein with a molecular weight of 15,000 and like IL-2 of other species, it is required for the clonal expansion of activated T cells with specific cytotoxic, helper and suppressor functions and with natural killer cell activity.[80-82]

The establishment of a normal immune response after antigen challenge depends upon an appropriate communication between various immunocompetent cells. This can be accomplished either by direct cellular interactions or via soluble molecules, the so-called cytokines (monokines and lymphokines, etc.) secreted by these cells. The abnormality of the regulatory (suppressor) T-cell function has often been implicated in the underlying immunopathogenesis

causing augmented IgE antibody production in atopic patients.[83,84] Thus, we felt it would be interesting to study the production of and responsiveness to IL-2 of lymphocytes of allergic patients, especially the effects of hyposensitization.

In these studies, IL-2 was produced by stimulating 4×10^6 MNC/ml with 1% PHA for 36 hours or with house dust (20X, final dilution) for five days. The IL-2 activities of the supernatants were titrated on an IL-2-dependent murine cytotoxic T cell line (CTLL, 1.5×10^4 cells/ml). The highest dilution titer which intercepted the 50% maximal proliferation was defined as the units of IL-2 in the tested sample. For responsiveness assay, lymphocytes were activated for 5 days with medium alone, with medium containing 1% PHA or with medium containing 20X diluted house dust. The cells were then washed and resuspended at a concentration of 1.5×10^5 viable cells/ml. One hundred μl of this cell suspension was added to each well in 96-well culture trays, followed by addition of 100 μl of medium containing Jurkat cell line-derived IL-2. The mixtures were cultured for 18 hours, pulsed with 1 μCi ^{3}H-thymidine for 6 hours and then harvested. Radioactivity was counted using a beta scintillation counter.

The results showed: (1) when stimulated with specific allergen (house dust), but not with PHA, the lymphocytes from newly diagnosed patients produced a much greater amount of IL-2 than did those from both hyposensitized patients and normals[85] and (2) the responsiveness to IL-2 of activated lymphocytes from hyposensitized patients was much more vigorous than that from both new patients and normals.[85] The decreased production of IL-2 by lymphocytes from hyposensitized patients corresponds to the diminished lymphoproliferative response of OKT4 cells to antigen stimulation[77] and indicates a state of diminished hypersensitivity after hyposensitization. The amplitude of T-cell immune response depends upon the interaction between IL-2 and IL-2 receptors (IL-2 R) on the T-cell surface.[82] In order to explore the mechanism(s) for enhanced responsiveness to IL-2 of activated lymphocytes from hyposensitized patients, the expression of IL-2R on T cells of normals, new patients and hyposensitized patients was studied using both fluorescence isothiocyanate-conjugated anti-Tac and ^{125}I-labelled anti-Tac (a gift from Dr. T.A. Waldmann[86]). The preliminary results showed that the expression of IL-2R on T cells of hyposensitized patients increased, although the changes, when compared to the expression of normals and new patients, were not statistically significant.[87] Thus, hyposensitization is able to modulate the immunological functions of T cells with respect to production of IL-2, responsiveness to IL-2 and expression of IL-2R. It is speculated that such alterations may partly explain the immunological changes occurring in patients receiving long-term hyposensitization.

In order to explore the possiblity of using IL-2 clinically for the treatment of allergic diseases, the effect of IL-2 on the generation of suppressor cell activity was studied.[78] The IL-2 was not only able to increase the number of viable cells in the allergen-stimulated MNC population from hyposensitized patients, but also to enhance their allergen-specific suppressor cell activity. Thus, in order to turn off the ongoing specific IgE antibody production, the infusion of allergen-specific suppressor cells which have been stimulated by allergen and expanded by IL-2 *in vitro* may be worth trying in the treatment of allergic diseases in the future.

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