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The Modulatory Effect of Immunoglobulin G on the CD_{23} and HLA-DR Expression and Cytokine Production in Different Groups of Asthmatic Patients

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SUMMARY The therapeutic effect and mechanism of action of immunoglobulin G (IgG) on bronchial asthma are not defined. Recently, it has been proposed that mononuclear cell (MNC) infiltration in the airway plays a role in the pathogenesis of asthma. In this study, we evaluated the effect of IgG on the cell receptor expression and cytokine production of MNC from two groups (young atopic and old non-atopic) of stable asthmatic patients. MNCs from both asthmatic patients and normal healthy individuals were obtained after Ficoll-Hypaque separation. Cells were cultured in serum free AIM-V medium, with or without phytohemagglutinin (PHA, 5 μg/ml) and IgG (100 μg/ml). After culture, MNCs were harvested and stained with monoclonal antibodies for HLA-DR (Ia), CD_{23} and CD_3. MNC supernatants were collected for IL-2 and IL-4 measurement. The results showed an enhancing effect of IgG on young atopic MNC proliferation when stimulated with PHA. The production of IL-2 and IL-4 from MNCs were significantly higher in old non-atopic asthmatics after PHA stimulation. The CD_{23}, but not HLA-DR, expression on CD_3 positive T cells and cytokines (IL-2 and IL-4) production were increased by IgG when stimulated with PHA in young atopic asthmatics. To the contrary, the effect of IgG on PHA stimulated MNC proliferation, CD_{23} and HLA-DR expression on CD_{23} positive T cells in old non-atopic asthmatics were trivial. Only IL-4 production can be significantly inhibited by IgG. These results suggested that the therapeutic effect of IgG on asthmatics might be variable in different groups of asthmatics. The higher amount of IL-2 and IL-4 production and significantly reduction of IL-4 by IgG might be relevant to the therapeutic effect of IgG on old non-atopic asthmatics.

Asthma is a common disease with heterogeneous course and pathogenesis. The majority of patients get relief with pharmacologic substance or avoidance of inciting factors. However, a subset of asthmatics suffer from a severe, chronic and debilitating disease which necessitates more aggressive therapy. Among the commonly prescribed drugs for the severe asthmatic are systemic corticosteroids. They are often effective, but prolonged use leads to a myriad of side effects, including hirsutism, cushingoid appearance and weight gain. Especially worrisome in the aged patients are osteoporosis, immune suppression, diabetes mellitus and hypertension.

In order to minimize steroid side effects in these steroid-dependent asthmatic patients, therapy with other remitting agents has been attempted. Gold injections,[1] low-dose methotrexate,[2] cyclosporine[3] and intravenous immunoglobulin G (IVIG),[4] having been employed as potential steroid-sparing agents, are all associated with toxic side effects and may not be ideal for use in aged intrinsic asthmatic population.

It has been reported that both T- and B-cell compartments show deficiencies in activation in the aged population.[5] IgG, IgA and IgM production tends to be lower in old than in young donor pokeweed mitogen (PWM)-stimulated cell culture.[6] Immunoglobulin therapy has been shown to be effective in a number of collagen-vascular diseases,

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especially where there is a large inflammatory component. Its efficacy in Kawasaki's disease,[7] polymyositis,[8] or systemic juvenile rheumatoid arthritis[9] may be explained partially by inhibition of release of important cytokines and enables significant reduction in dose of corticosteroid,[8,9]. In bronchial asthma, the precise mechanism of anti-inflammatory effect of immunoglobulin has not yet reported.[10,11]

It has been reported that patients with acute severe asthma have significant increases, compared with control subjects, of three surface proteins associated with T lymphocyte activation: interleukin-2 receptor (IL-2R); class II histocompatability antigen (HLA-DR); and "very late activation" antigen (VLA-1).[12] IgE binding receptors (CD_{23}) also appears to play a key role in the regulation of IgE synthesis as allergic individuals have shown to have increased IgE binding receptors.[13] It has also been reported that IgE synthesis can be regulated by IL-4. The present study investigated the immunomodulatory effect of IgG on asthmatic monomuclear cells (MNCs) by studying cytokine production, cell activation markers (HLA-DR), and IgE binding receptor (CD_{23}) expression. This study can potentially detail a mechanism of intravenous immunoglubulin G (IVIG) therapy, and also provide a possible alternative therapy in the intractable steroid dependent asthmatics.

MATERIALS AND METHODS

Materials

The Ficoll-Hypaque was obtained from Pharmacia Fine Chemicals AB, (Uppsala, Sweden). Human-IgG and paraformaldehyde were purchased from Sigma Chemical Co. (St. Louis, MO, USA). AIM-V medium and Hanks' balanced salt solutions (HBSS) were purchased from Gibco Biological Co. (Grand Island, NY, USA). Purified-phytohemagglutinin (PHA) was obtained from Wellcome Diagnostics (Dartford, England). Monoclonal antibodies (MAb) reactive with MNC surface antigen, CD_3, CD_{23}, and HLA-DR (MHC Class II) were purchased from Becton Dickinson Immunocytometry systems (California, USA). The radioisotope [^{3}H]-thymidine (6.7 Ci/mmole) was purchased from New England Nuclear Corp (Boston, MA, USA). Both IL-2 and IL-4 ELISA kits were purchased from Research and Diagnostics Systems, Inc. (MN, USA). All other materials used were of the highest quality commercially available.

Patient population

Human subjects between 18 and 60 years of age were recruited from the allergic clinic of Veterans General Hospital-Taipei and from the staff of the Hospital. Bronchial asthma, diagnosed by using pulmonary function test, was defined by 20% reversability of FEV_1 and/or $FEF_{25-75\%}$ after beta-2 agonist inhalation. Young atopic asthma was defined as those whose age was below 40 (28 ± 5 years old) and skin test positive to multiple aeroallergen. Old non-atopic asthmatic was defined as those whose age was above 60 (65 ± 4 eyars old) and skin test negative to common aeroallergen.

Isolation and culturing of mononuclear cells

Human MNCs were isolated from the heparinized venous blood of normal and healthy adult and asthmatics as previously described.[15] Briefly, peripheral blood was layered on a Ficoll-Hypaque cushion and was centrifuged at $740 \times g$ for 25 minutes at room temperature. The MNCs at the interface were washed three times with Hanks' balanced salt solution (HBSS). The cells were cultured in TC tube (Greiner Lubortechnic, Germany) in a humidified atmosphere containing 5% CO_2 at 37°C for two days. The culture medium consisted of AIM-V medium supplemented with 100 μg/ml streptomycin and 100 IU/ml penicillin. In this study, 5 μg/ml phytohemagglutinin and 100 μg/ml IgG were added to culture medium respectively. All cells were cultured at a concentration of 5×10^6 cells/ml. After two days culture, supernatant was collected and stored in -70°C prior to cytokine assay.

Mononuclear cell proliferation assays

After two-day incubation, human MNCs were cultured with [^{3}H]-Thymidine (5 μCi/ml) in 96-well, flat-bottom microtitreplates (Corning, New York) at 37°C (5% CO_2) for six hours. At the end of incubation, multiwell plate was chilled and harvested onto glass fiber filter (Whatman, England). Incorporation of radioactivity was measured by liquid scintillation analyzer (Packard-1500, Canberra Corp, Frankfurt). The results are expressed as the mean counts per minute. All determinations were made in triplicates.

Immunofluorescent staining and flow cytometry analysis

The details of immunofluorescent staining and FACS analysis have been published previously.[15] Briefly, MNCs were incubated with fluorescein isothiocyanate (FITC)-conjugated anti-CD_3 and phycoerythrin (PE)-conjugated anti-CD_{23} MAbs or with FITC-conjugated anti-CD_3 and PE-conjugated anti-HLA-DR MAbs for 30 minutes at 4°C. The cells were analyzed by a Becton Dickinson FACS-scan system flow cytometry (Becton Dickinson Worldwide Inc., CA, USA) operating at a laser wave length of 488 nm and power output of 15 milliwatts. In each experimental condition, a total of 2,000 cells were analysed.

ELISA for IL-2 and IL-4

IL-2 and IL-4 were measured by commercial available ELISA kits, using mouse monoclonal antibodies recognizing different epitopes of the cytokine molecules. The lowest detectable ranges of these kits were 6.0 pg/ml for IL-2 and 3.0 pg/ml for IL-4.

Statistical analysis

The results were analysed by paired student's *t* test to compare the same groups of individuals under different treatment; and by non-paired student's *t* test to compare the differences between young atopic and old non-atopic asthmatics. Friedman Anova was applied to analyse the effect of IgG on MNC proliferation in the presence of PHA.

RESULTS

The effects of IgG on MNC proliferation

MNC proliferation could be enhanced by IgG at the concentration of 100 μg/ml in the presence of PHA (Fig. 1A). The effect of IgG on unstimulated MNC was negligible. IgG, at the concentration of 100 μg/ml, could significantly enhance PHA-stimulated MNC proliferation in young atopic asthmatics; however, the enhancing effects in old non-atopic asthmatics was negligible (Fig. 1B).

The effects of IgG on CD_{23} and HLA-DR receptor expression

In the study of receptor expression on CD_3 positive T cells, both CD_{23} and HLA-DR could be enhanced by PHA in both group of asthmatics.

The IgE binding receptor, CD_{23}, was significantly enhanced after PHA stimulation ($12.6 \pm 0.1\%$ *vs* $59.4 \pm 0.1\%$ in young atopic group, $10.2 \pm 0.1\%$ vs $61.6 \pm 0.1\%$ in old non-atopic group; $p < 0.001$). When asthmatic MNCs were co-cultured with PHA and IgG, CD_{23} could be further significantly en-

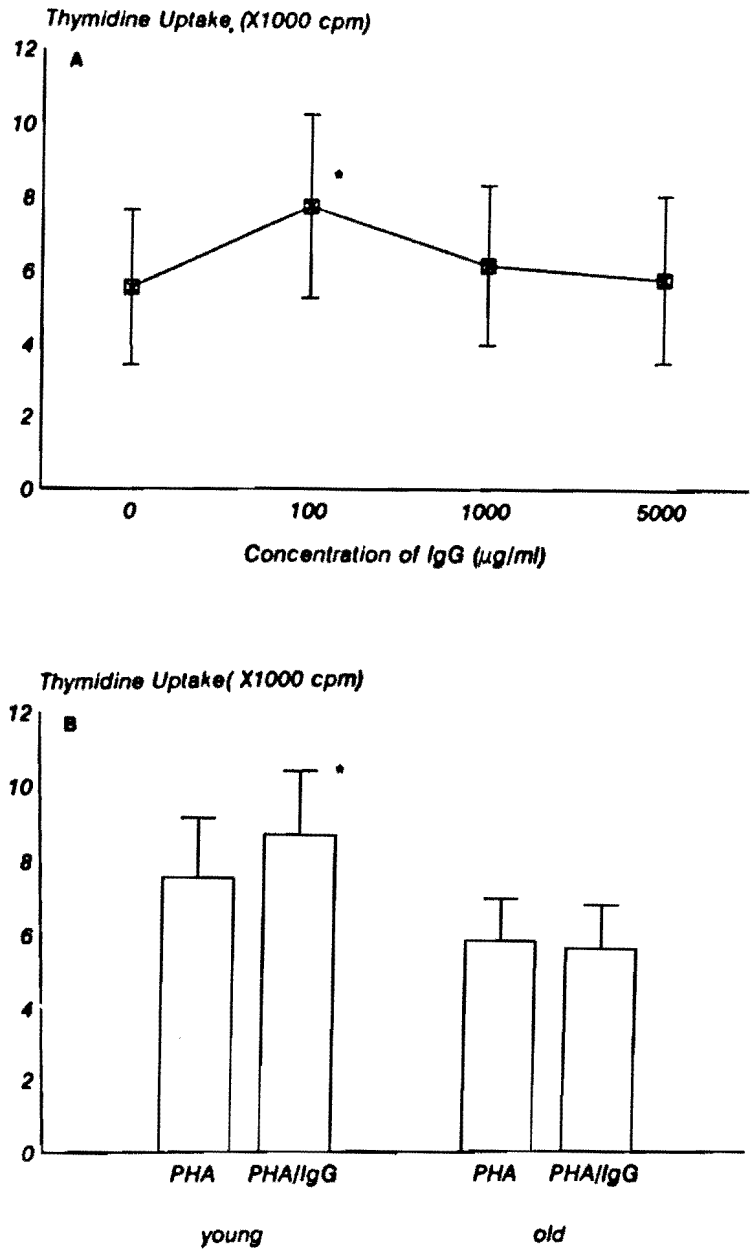

Fig. 1 The effect of IgG on MNC proliferation from asthmatics and normal subjects. MNCs from normal subjects were cultured with different concentration of IgG in the presence of PHA for 2 days. MNC proliferation was expressed as [^{3}H]-TdR incorporation. Each point represents mean ± SEM of 4 experiments, $^*p < 0.05$ when compared to control (A). The effect of IgG on PHA-stimulated MNC proliferation in young atopic and old non-atopic asthmatics. Each column represents mean ± SEM of 10 experiments (B). *Significant difference between PHA-stimulated and PHA/IgG stimulated MNC prolieration in young atopic asthmatics ($p < 0.05$).

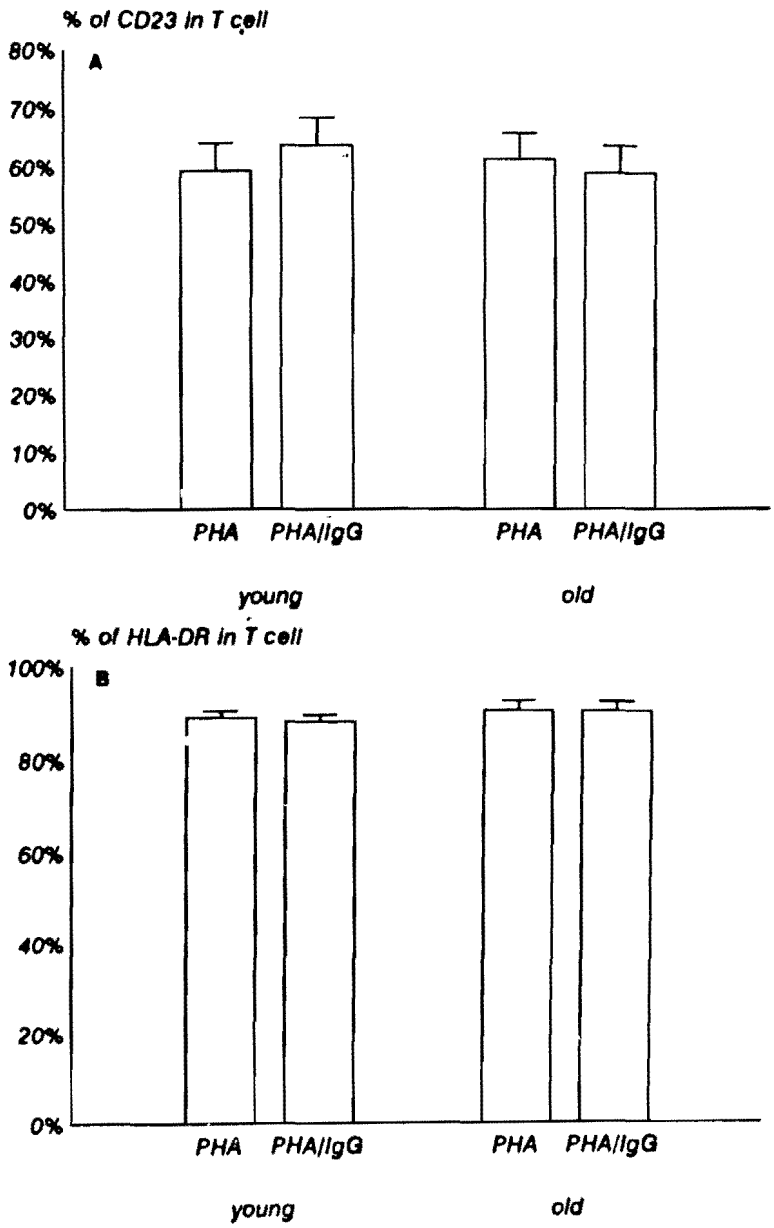

Fig. 2. The effect of IgG on the CD_{23} and HLA-DR expression on CD_3 positive T cells from asthmatics. Asthmatic MNCs (young atopic and old non-atopic), having been cultured with PHA and IgG, were double-stained for studying their CD_{23} and HLA-DR expression on CD_3 positive T cells. 2,000 cells were anlyzed in each case using FACs-scan analyser. Data were expressed as percent positive CD_{23} on CD_3 positive T cells (A) and percent positive HLA-DR on CD_3 positive T cells (B). Each column represents mean ± SEM of 10 experiments. $^*p < 0.05$ when compared with PHA-stimulated MNCs in young atopic asthmatics.

hanced by IgG in young atopic asthmatics ($P < 0.01$). The effects of IgG on CD_{23} expression were trivial in old non-atopic subjects (Fig. 2A).

When MNCs were stained with HLA-DR MAb, HLA-DR antigen could be significantly enhanced by PHA in both group of asthmatics ($25.4 \pm 0.02\%$ *vs* $89.1 \pm 0.01\%$ in young atopic group, $27.8 \pm 0.02\%$ vs $90.7 \pm 0.01\%$ in old non-atopic group; $p < 0.001$). But this enhancement of HLA-DR by PHA could not be suppressed by IgG (Fig. 2B).

The effect of IgG on IL-2 and IL-4 production

When MNCs cultured with PHA, there were significant higher production of IL-2 and IL-4 in old non-atopic asthmatics than the young atopic group (Fig. 3).

When MNCs cocultured with PHA and IgG, both IL-2 and IL-4 can be significantly enhanced by IgG in the young atopic asthmatics. To the contrary, the cytokines production were reduced by IgG in old non-atopic group. However, only IL-4 production was reduced significantly by IgG (Fig. 3).

DISCUSSION

This study demonstrated that the modulatory effect of IgG on MNC cytokine production and receptor expression varied in different asthmatic groups. There was a discrepancy between young atopic and old non-atopic asthmatics. Immunoglobulin G not only enhanced PHA-stimulated MNC proliferation but also enhanced CD_{23} expression and cytokine (IL-2 and IL-4) production from PHA-stimulated MNCs in young atopic asthmatics. In contrast, MNCs from old non-atopic asthmtics were more resistant to IgG *in vitro*. Only IL-4 production was reduced by IgG in the PHA stimulated MNC culture.

Several potential immunoregulatory effects of IgG on allergic

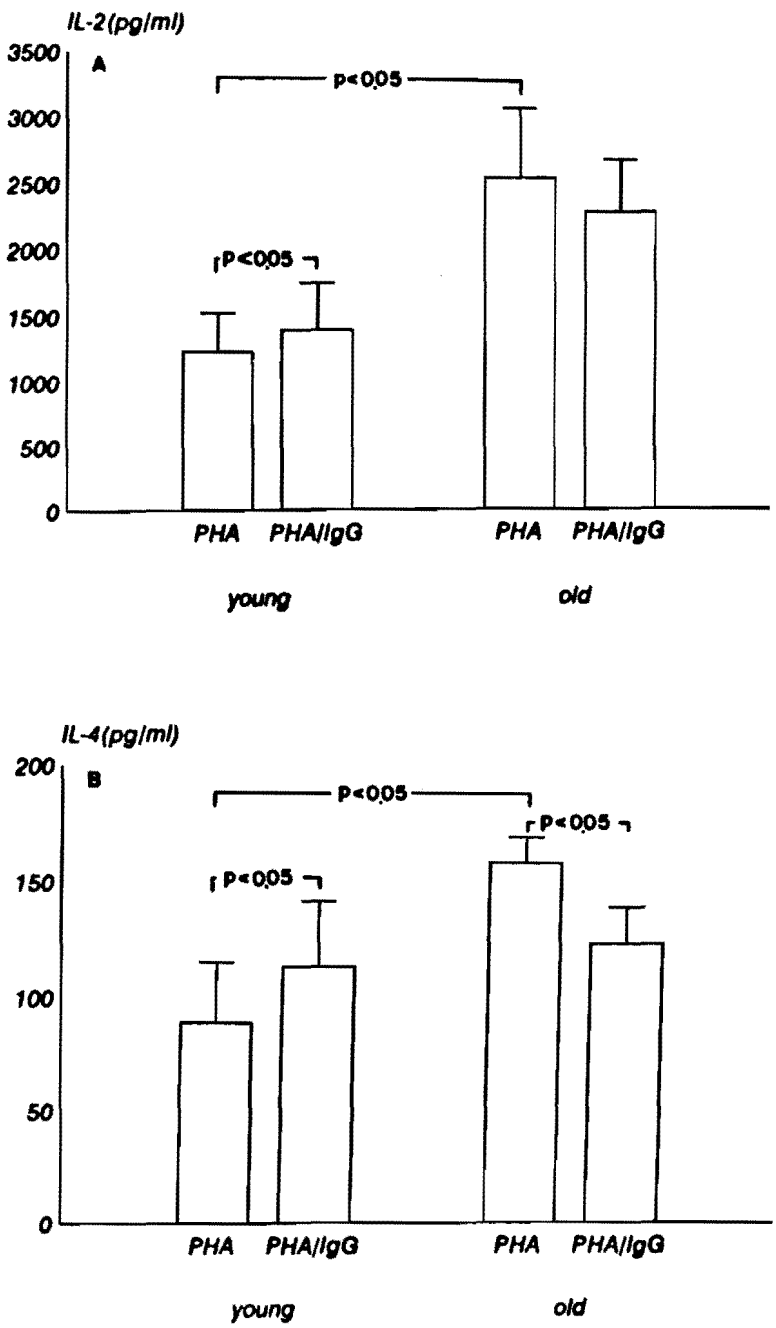

Fig. 3. The effect of IgG on IL-2 and IL-4 production from young atopic and old non-atopic asthmatics. MNCs (2×10^6/ml), having been cultured with PHA with or without IgG, supernatants were collected for IL-2 and IL-4 determination. Each column represents mean ± SEM of 10 experiments.

diseases have been raised including blocking antibody, inhibition of antigen presentation, inhibition of cytokine production by T cells and IgE production by B cells.[11] In this study, we demonstrated that IgG could selectively inhibit IL-4 production in old non-atopic asthmatics. This result indicated that old non-atopic asthmatic MNCs were more susceptible to suppression by IgG than those of young atopic asthmatics.

The low affinity IgE receptor CD_{23} has been described as a 45-kilo-dalton protein found on lymphocytes and macrophages.[16] High level of CD_{23} on cells and in the serum have been found in diseases associated with elevated IgE and activated B cells.[17,18] A common feature of these CD_{23} is their increased expression on activated cells to serve several biological activities in these cells. In our study, CD_{23} could be up-regulated by IgG in young atopic asthmatics; thus, IgG might have some effect on B cell activation and IgE synthesis. This might be important in bronchial asthma since increasing the IgE and its affinity receptors might cause the release of a variety of inflammatory mediators such as histamine and leukotrienes, and then induce allergic inflammation in young atopic asthma.[19] Despite IgG may up-regulate CD_{23} expression on T cells of young atopic asthmatics, which may enhance B cell activation and IgE synthesis. Several other mechanisms of action of IVIG in allergic disorders have been reported.[14] IVIG may serve as a blocking antibody, alterate T- or B-cell function and modulate antigen presenting cell activity. Whether IVIG worked predominantly on T cells of young atopic asthmatics is still uncertain, this cannot be clear until all potential target cells had been studied together.

T cell activation requires the recognition of processed antigen in

association with class II major histocompatibility antigen (HLA-DR) on the surface of antigen presenting cells (APC).[20]. Since lymphocyte activation and cytokine generation has been demonstrated to play an important role in the pathogenesis of bronchial asthma,[21] the trivial effect of IgG on HLA-DR antigen expression on both group of asthmatic CD_3 positive T cells further suggests that the modulatory effect of IgG on allergic inflammatory reaction might not through HLA-DR antigen expression on activated T cells.

It has been reported that patients with acute severe asthma have increases of three surface proteins associated with T lymphocyte activation: IL-2R, VLA-1 and HLA-DR. The percentages of IL-2R positive and HLA-DR positive lymphocytes tend to decrease as the patients become clinically improved.[12] In our study, both IL-2 and IL-4 production were increased by IgG in young atopic asthmatics, suggesting the beneficial therapeutic effect of IgG on young atopic asthmatics might not through these cytokine production. The significantly higher amount of IL-2 and IL-4 production in old non-atopic asthmatics further suggested that there were more T cells being activated by PHA in old non-atopic asthmatics. The higher levels of IL-2 and IL-4 produced by MNCs of aged asthmatics after PHA stimulation also indicated that there were more inflammatory reaction and T cell activation in aged asthmatics. Some studies have demonstrated that chronic asthmatics have more tissue inflammation: a long-sustained inflammation of the bronchi leading to a variable degree of bronchial hyperreactivity and tissue destruction after some decades of chronic asthma have been described.[22-24] The discrepancy of the modulating effect of IgG on different group of asthmatics (upregulating in young atopic group and down regulating in old non-atopic group) might be due to the relative difference of the clinical status. The precise mechanism of IgG in different groups of asthmatics cannot be explored until the allergen specific T cell clone from each age groups had been established.

In conclusion, the immunomodulatory effect of IgG on T cell expression of CD_{23} and cytokine production was variable in different asthmatic status which may have some relevance to the therapeutic effect of IgG. This study could be used to explain favorable therapeutic effect of IVIG in aged asthmatics, however, its working mechanism in young atopic asthmatics needed to be further clarified.

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