Acta Derm Venereol (Stockh) 1989; Suppl 144: 127–130

In Vitro Generation of IFN-gamma in Relationship to in vivo Concentration of IgE and IgG Subclasses and $Fc_\varepsilon R_L$/CD23 Positive Circulating Lymphocytes in Patients with Severe Atopic Dermatitis (AD)

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In fifteen patients with severe atopic dermatitis (AD) and ten healthy controls we investigated the in vitro generation of IFN-gamma and analysed the number of $Fc_\varepsilon R_L$/CD23 (low affinity Fc receptor for IgE) positive lymphocytes. We found a significantly impaired capacity to secrete IFN-gamma after PHA-stimulation compaired to controls in a significant proportion of patients. Serum IgG_4 levels in patients were higher compared to controls. A significant portion of lymphocytes bearing the low affinity Fc receptor for IgE (CD23) was observed with the moab Tü1 in patients. Lymphocytes from healthy donors were completely negative or $< 2\%$ positive for Tü1. Despite small numbers of patients a significant correlation was found between IFN-gamma generation in vitro and IgE serum concentration in patients, whereas the IFN-gamma generation and IgG_4 concentration were negatively correlated in the patient group. The number of $Fc_\varepsilon R_L$/CD23 positive lymphocytes in patients was positive correlated with the serum IgG_4 and IgE concentration and negative correlated with IFN-gamma generation in vitro. Our data suggest that a possible dysregulation of IFN-gamma, Interleukin 4 or other lymphokine production may be related to increased IgE and IgG_4 production.

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In contrast to normal controls peripheral blood mononuclear cells (PBMC) from patients with AD spontaneously synthesize detectable amounts of IgE in vitro. Furthermore abnormal suppressor-cell activity is well documented and IgE-specific helper factors released by T-cells from AD patients are involved in the pathogenesis of the disease (1). Recently, the low affinity Fc-receptor $Fc_\varepsilon R_l$) for IgE normally present only on a minor portion of monocytes, B cells, eosinophils and platelets has been identified with an increased number on circulating monocytes, T and B cells in AD. Defranc et al. (3) have shown that Il4 is able to induce FcR_L/CD23 on B-cells and Snapper (4) found that Il4 may enhance IgE and IgG_1 production of B-cells and may induce MHC class II expression on resting B-cells. IFN-gamma has been found to inhibit I14-mediated FcR_L/CD23 expression on B-cells and enhancement of IgE production. We have investigated the in vitro IFN-gamma production and analyzed the relationship to IgE and IgG_4 concentration in vivo and the number of FcR_L/CD23 positive circulating cells in patients with AD.

SUBJECTS

Fifteen patients with AD (median age 23 years, range 15–35 years) and ten healthy controls (median age 21 years, range 18–25 years) were investigated. The clinical degree of AD was evaluated by the criteria of Hanifin & Rajka (5).

METHODS

In vitro IFN-gamma production was induced by stimulating PBMC with phythaemagglutinin (PHA) at a dose of 1% stock-solution (Gibco, Karlsruhe, FRG). Cultures were performed in 24-well Costar culture plates (Tecnomara, Fernwald, FRG) at 1×10^6/ml for a period of 5 days at 37°C, 5% CO_2. The daily production of IFN-gamma was measured by removing the supernatants of activated PBMC. Supernatants were stored at −20°C until assayed for their content of IFN-gamma. Human IFN-gamma was assayed by an immunoradiometric assay from Celltech (Slough, UK) after 24 and 48 h stimulation of cells. Serum IgE concentrations were determined by a solid phase radioimmunoassay (Phadebas IgE Prist, Pharmacia, Freiburg, FRG) and IgG-subclasses were measured by enzyme linked immuno sorbent assay (ELISA) utilizing monoclonal subclass specific antibodies (Bio-Makor, Rehovot, Israel) as described by Jeffries et al. (6). PBMC labeled with moab (Tü1) were developed with a FITC-conjugated $F(ab)_2$ rabbit anti-mouse Ig (Dianova, Hamburg, FRG) as second antibody and analyzed on a FACS IV. Student's *t*-test was employed for statistical comparison and for correlation analysis the Spearman correlation coefficient was used.

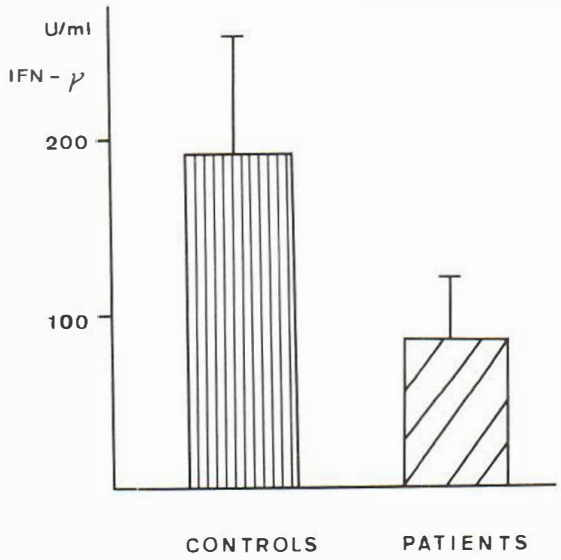

Fig. 1. IFN-gamma generation in vitro.

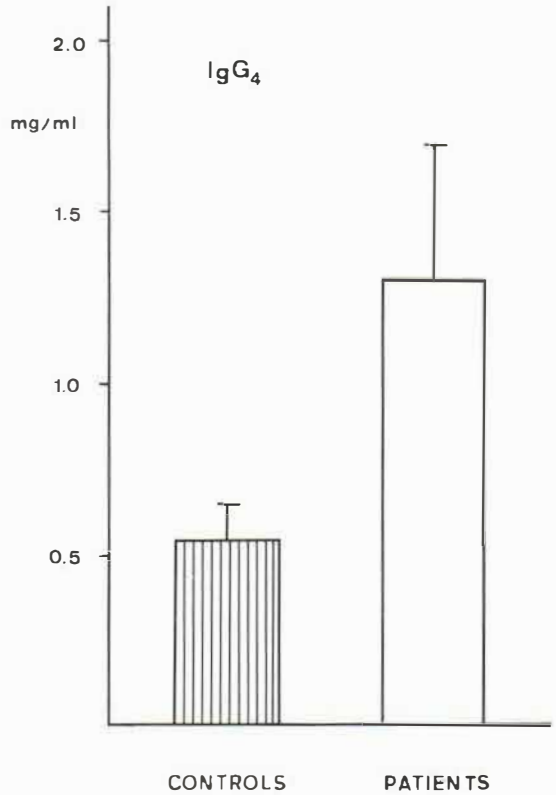

Fig. 2. IgG_4 serum concentration.

RESULTS

IFN-gamma generation by PBMC reached a maximum at 48 h after stimulation with PHA in AD and controls. IFN-gamma production in patients with AD (mean 90 ± 26 U/ml) was significantly lower ($p < 0.05$) than in controls (mean 193 ± 46 U/ml) (Fig. 1). The IFN-gamma levels in supernatants of unstimulated controls at 48 h were < 10 U/ml. The arithmetic mean of IgG subclass concentrations in controls did not differ significantly to WHO reference serum concentration (67/97). We found higher IgG_4 levels (mean 1.38 ± 0.40 mg/ml) in the patient group than in controls (0.59 ± 0.11), although this was not statistically significant due to low sample number ($n = 7$) (Fig. 2). Levels of IgG_1, IgG_2 and IgG_3 did not differ significantly from those of controls. In seven of eight patients a significant portion of Tü1-positive lymphocytes (range 2–10%) was observed, those of controls were completely negative or < 2%. Despite small numbers of patients a significant correlation was found between IFN-gamma generation in vitro and IgE serum concentration in vivo ($r = -0.66$, $p < 0.001$) (Fig. 3). The number of $Fc_\varepsilon R_L$/CD23 positive lymphocytes was positive correlated with serum IgG_4 concentration ($r = 0.97$, $p < 0.001$) (Fig. 4).

DISCUSSION

Our results demonstrate a highly significant relationship between in vitro and in vivo parameters in patients with AD. We found that PBMC of a significant proportion of patients have an impaired capacity to generate IFN-gamma after PHA-stimulation in vitro. IFN-gamma generation in vitro was significantly negative correlated with serum IgE concentrations in vivo. In agreement with other groups (6) we detected higher IgG_4 levels in sera of patients compared to controls.

Although the mechanism of IgG_4 elevation in AD is not clear, it has been suggested that it is raised due to prolonged exposure to an allergen which initiated an IgE response. Sherr et al. (1) have indicated a major role of helper factors released by activated T lymphocytes in the regulation of IgE secretion. Il 4 represents a T cell-derived lymphokine that enhances the secretion of IgG_1 and IgE and stimulates mast cell growth. Parkin et al. (8) suggested that the induction of $Fc_\varepsilon R_L$/CD23 on B cells seems to be specific for Il 4. Murine helper/inducer T cell clones are composed of at least two nonoverlapping subsets that can be distinguished on basis of their patterns of lymphokine secretion (9). The Th1 subset is able to produce Il2 and

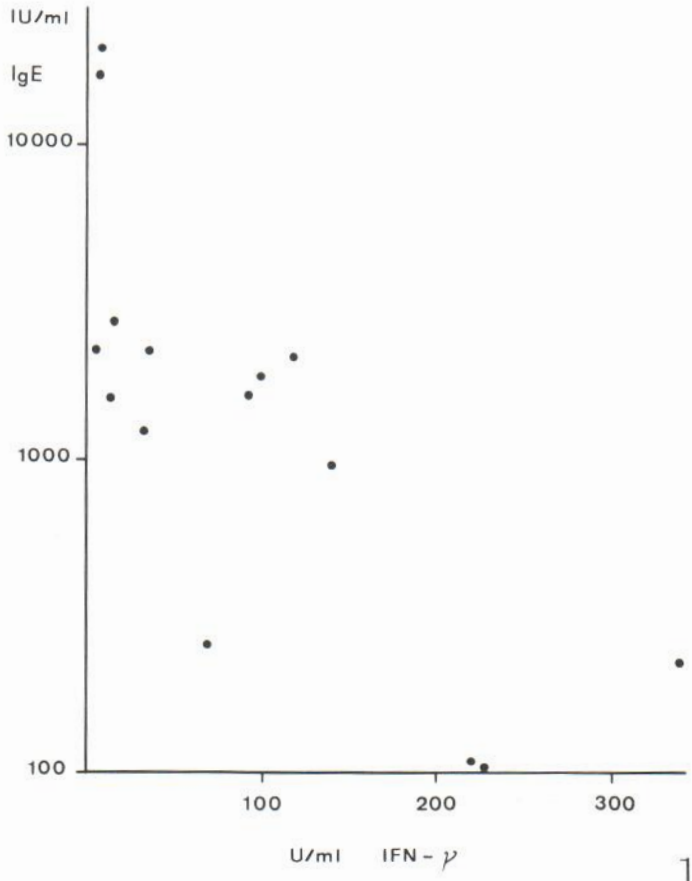

Fig. 3. Correlation between IFN-gamma generation in vitro and IgE serum concentration, $r = -0.66$; $p < 0.001$.

IFN-gamma in response to antigen receptor-mediated stimulation. The Th2 cell subset secretes Il 4 but not Il2. The T cell subset Th2 also secretes Il 5, which has been shown to have growth-promoting properties for eosinophils (10).

The selective activation of Th2 cells in vivo by certain allergic agents might be expected to increase $Fc_\varepsilon R_L$/CD23 expression, IgG_1 and IgE levels and the number of mast cells that bind IgE and mediate the allergic reaction. In addition Th2 cells may stimulate the growth of eosinophils via Il 5. Small quantities of IFN-gamma can totally inhibit the ability of Il 4 to stimulate B-cell growth, enhance IgG_1 and IgE production and the induction of $Fc_\varepsilon R_L$/CD23 on B cells (3). Our data suggest that the low IFN-gamma in vitro generation in a significant portion of AD patients may play a major role in the pathogenesis of increased IgE production and enhanced expression of $Fc_\varepsilon R_L$/CD23 lymphocytes. This is further confirmed by the observed correlation between IFN-gamma generation in vitro and serum IgE concentration in vivo.

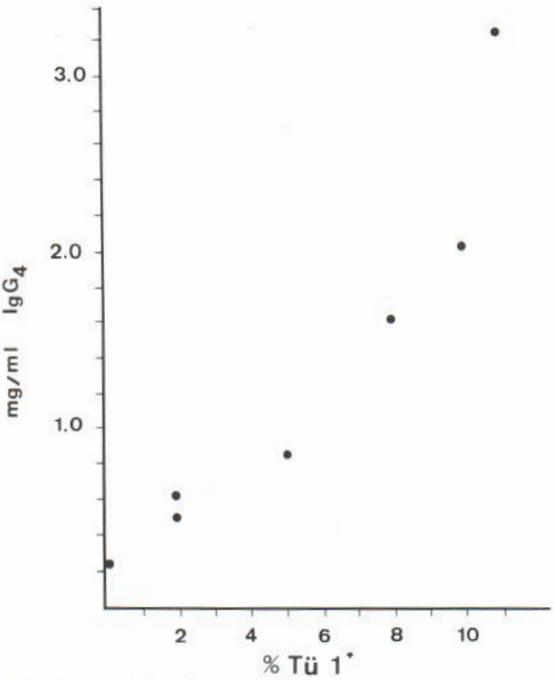

Fig. 4. Correlation between $Fc_\varepsilon R_L$/CD23 positive lymphocytes and IgG_4 serum concentration, $r = 0.97$; $p < 0.001$.

Studies of Strannegard (11) have indicated a defective capacity to generate interferons in response to viral antigens in AD. However, these and our results are in contrast to studies from Kapp et al. (12), who did not report any differences in IFN generation in AD patients in vitro. Interestingly, a portion of patients analyzed in their studies did not secret any or at least small levels of IFN-gamma in response to PHA which is actually in agreement with our present data. Furthermore in Kapp's study the IFN-gamma generation was measured using a bioassay while we used a highly sensitive IRMA-test specific for IFN-gamma in our experiments. Taken together the results of Kapp and our group suggest that defective IFN-gamma generation is not a primary defect in all AD patients, but is present in a subgroup of patients and seems to be an important factor in the pathogenesis of AD. Furthermore, the possible relation of in vitro parameters to the patients clinical status (all had severe AD) and course has to be elucidated in further studies.

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