

Effect of interferon- γ on HLA Class 2 expression by antigen-presenting cells inducing *Dermatophagoides farinae*-specific interleukin-2 responsiveness of lymphocytes in asthmatic children

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As previously reported, antigen-specific IL-2 responsiveness of lymphocytes from patients with bronchial asthma was induced by stimulation with *Dermatophagoides farinae*, antigen-specifically, in the context of HLA-DQ antigen. We analysed the antigen-presenting processes in the induction of IL-2 responsiveness. Non-adherent responder cells cultured with Df-pulsed autologous adherent cells acquired IL-2 responsiveness, which decreased after prolonged culture of adherent cells for 72 h before recombination with fresh autologous non-adherent cells. HLA-DQ expression on cultured adherent cells also decreased in parallel with the reduction of their ability to induce IL-2 responsiveness. However, the treatment of adherent cells with IFN- γ restored the expression of these HLA-Class 2 antigens mainly on CD14+ antigen-presenting cells (monocytes), but not on CD20+ cells (B cells), which overcame the decrease of Df-induced IL-2 responsiveness during prolonged culture. These data suggest that IFN- γ potentiates the up-regulation of Df-specific IL-2 responsiveness, which is likely to depend on the restoration of HLA-DQ expression on monocytes.

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Induction of interleukin-2 (IL-2) responsiveness after antigen stimulation appears to be essential for T cells to carry out several kinds of immunologic functions (1, 2, 3). The presence of allergen-sensitized lymphocytes in allergic patients has been demonstrated by *in vitro* proliferative responses and IL-2 production by lymphocytes stimulated with antigen. Children with bronchial asthma are usually sensitized with *Dermatophagoides farinae* (Df) antigen.

We previously reported an allergen-specific acquisition of IL-2 responsiveness in patients sensi-

tized with Df after *in vitro* restimulation of peripheral blood lymphocytes (PBL) with allergen (4, 5). This phenomenon was not observed when the lymphocytes of patients allergic to Df alone were stimulated with other antigens, such as ovalbumin (OVA), which suggests the antigen-specificity of this reaction. Moreover, we reported that this induction of Df-specific IL-2 responsiveness is mediated by antigen-presenting cells in the context of appropriate human histocompatibility leukocyte antigen (HLA) Class 2 antigen (5). However, expression of HLA-Class 2 antigens on macrophages is diminished after prolonged *in vitro* culture. In contrast, increased expression of these antigens is observed after incubation of macrophages in media containing interferon- γ (IFN- γ). The present study was undertaken to evaluate in detail the functional expression of HLA-class II antigen on

Abbreviations:

PBMC: peripheral blood mononuclear cells; IL-2: interleukin-2; IFN- γ : interferon- γ ; Df: *Dermatophagoides farinae*; OVA: ovalbumin; HLA: human histocompatibility leukocyte antigens; PBS: phosphate-buffered saline.

the antigen-presenting cells involved in antigen-specific IL-2 responsiveness.

Materials and methods

Peripheral blood mononuclear cells (PBMC) were obtained from patients with bronchial asthma diagnosed by clinical features and skin tests.

Preparation of macrophage-enriched fraction and T cell-enriched fraction

PBMC were separated from heparinized peripheral blood by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density-gradient centrifugation (6). To obtain macrophage-enriched adherent cells, PBMC suspended in RPMI 1640 medium (GIBCO, Life Technologies, Inc., NY) supplemented with 10% human AB serum were incubated for 2 hours in sterile plastic Petri dishes (100 mm tissue culture dish, No.25020, Corning Glass works, Corning, NY). T cell-enriched non-adherent cells were removed by three washings, and the adherent cells were collected by removing them from the plastic with a rubber policeman (7).

Assay for IL-2 responsiveness

In brief, mononuclear cells, 5×10^5 , were added to Df (Torii & Co., Tokyo, Japan) antigen and suspended in 0.5 ml of RPMI 1640 medium containing 40 µg/ml of gentamycin and 10% heat-inactivated human pooled serum in a culture tube (Falcon Plastics, Becton Dickinson Labware, Lincoln Park, NJ, 2054). In most experiments, we added 10 µg/ml Df to the culture, since this concentration was found to be optimal for the induction of IL-2 responsiveness. After culture at 37°C for 6 days, the cells were washed three times to remove antigen, readjusted to 2×10^5 in 200 µl of medium, and cultured for 3 more days in the presence of 0.1 unit/200 µl recombinant IL-2 (rIL-2) (Takeda, Osaka, Japan), since this concentration was found to be optimal for the stimulation of IL-2 responding cells. The number of viable cells was scored blindly by the trypan blue exclusion staining method. Cell viability was greater than 90% in all tests. IL-2 responsiveness in the present study was evaluated as a stimulation index (SI) which was calculated as follows:

IL-2 responsiveness (SI) =

$$\frac{\text{viable cell number with IL-2}}{\text{viable cell number without IL-2}}$$

To evaluate the ability of adherent cells to present

Df-antigen for the induction of IL-2 responsiveness, antigen-presenting cells prepared by plastic adherence were pulsed with Df antigen at 37°C for 2 h. 1×10^5 /ml of these Df-treated cells were added to 4×10^5 /ml non-adherent cells, and their capacity to induce Df-specific IL-2 responsiveness was evaluated (5).

Treatment of adherent cells with interferon-γ

Adherent cells were precultured with serial dilutions of recombinant INF-γ (rINF-γ) (Shionogi, Osaka, Japan) for 1 to 4 days and washed three times. After being pulsed with 10 µg/ml Df, these cells were recombined with non-adherent cells to evaluate IL-2 responsiveness.

FACS analysis of HLA-class II antigen expression

To elucidate the role of HLA-class II antigen on antigen-presenting adherent cells, adherent cells were precultured for 24 to 96 h and then added to 25 µl of fluorescein isothiocyanate (FITC)-conjugated monoclonal antibodies to various HLA-class II antigens, including Leu 10 (Becton Dickinson, Rutherford, NJ), OKIa1 (Ortho Diagnostics Systems) and HLA-DP (Becton Dickinson). After these cells had been incubated at 4°C for 30 min, they were washed three times with PBS and adherent cells stained with those agents were quantified on a FACS 440 instrument (Becton Dickinson Electronics Laboratories, Mountain View, CA).

Furthermore, to compare the IFN-γ-induced HLA-class II expression on macrophages and on B cells, T cells were removed by rosetting of PBMC with sheep red blood cells (SRBC) treated with 2-aminoethylisothiuronium bromide (AET, Sigma Chemicals Co, St. Louis, Mo) (8). SRBC-rosette-negative, non-T cells (5×10^5) were cultured for 2 days with or without rINF-γ in RPMI 1640 medium supplemented with 10% FCS. They were then washed three times with PBS to be tested for the expression of HLA-class II antigen on B cells or on macrophages. 25 µl of FITC-conjugated monoclonal anti-HLA-DR antibody (Becton Dickinson, Mountain View, CA) or Leu 10 (HLA-DQ) antibody (Becton Dickinson, Mountain View, CA) and phycoerythrin (PE)-conjugated Leu 16 (CD20) (Becton Dickinson, Mountain View, CA) or Leu M3 (CD14) (Becton Dickinson, Mountain View, CA) monoclonal antibody were added to the cells and allowed to react at 4°C for 30 min. The cells were washed three times with PBS and their expression of HLA-class II antigens on B cells or on macrophages was quantified on a FACS 440. In the experiments identifying HLA-DP antigen, the cells were initially made to

Table 1. Interferon- γ modulates the ability of APC to induce IL-2 responsiveness

Treatment of APC with IFN- γ (4days)	Induced-IL2 responsiveness
IFN- γ (u/ml)	SI (mean $\pm$ SE)
none	1.39 $\pm$ 0.22
32.5	2.04 $\pm$ 0.02
125	2.56 $\pm$ 0.17
500	2.27 $\pm$ 0.17

Results are expressed as mean $\pm$ SE, because these experiments were performed in triplicate with cells of a single allergic donor. APC: antigen-presenting cell, IL-2: interleukin-2, IFN- γ : interferon- γ , SE: standard error, SI: stimulation index.

react with anti-HLA-DP monoclonal antibody (Becton Dickinson, Mountain View, CA) and then incubated with the F(ab')₂ portion of FITC-conjugated goat anti-mouse IgG (Tago, Burlingame, CA) and extensively washed to remove free antibody thoroughly before their binding to PE-conjugated anti-CD20 or anti-CD14 antibody.

Results

Up-regulating effect of IFN- γ on induction of IL-2 responsiveness

To determine the optimal concentration of rIFN- γ , graded doses of rIFN- γ were added to the preculture of adherent cells. As the optimal concentration of rIFN- γ was 125 U/ml (Table 1), this concentration of rIFN- γ was used in all experiments afterwards.

Df-specific IL-2 responsiveness decreased as the culture period of adherent cells was prolonged. But when adherent cells were precultured with

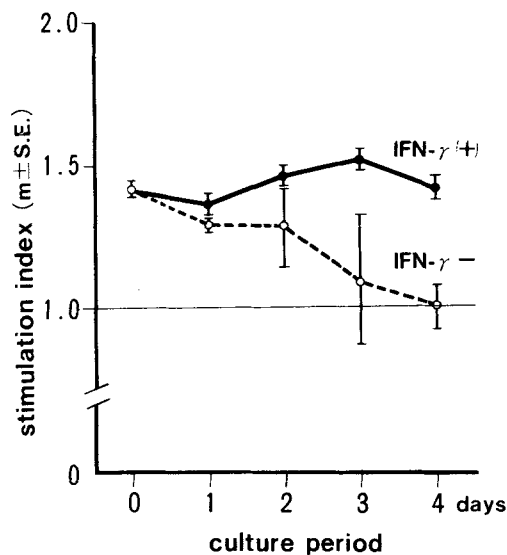


Fig. 1. IFN- γ is capable of improving the decreased function of adherent cells in culture for Df-induced IL-2 responsiveness of lymphocytes.

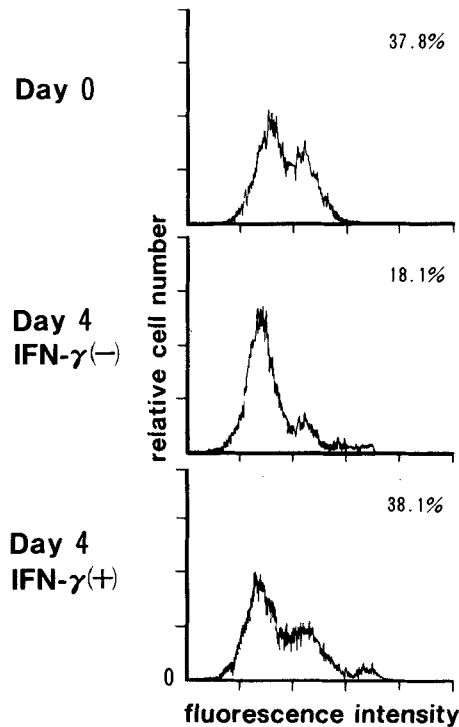


Fig. 2. HLA-DQ expression of adherent cell had decreased on day 4, but improved on addition of IFN- γ .

rIFN- γ , their ability to induce IL-2 responsiveness was maintained, and was equivalent to that of freshly separated adherent cells (Fig. 1).

Effects of IFN- γ on HLA-Class 2 antigen expression of cultured adherent cells

Fig. 2 shows the FACS pattern of DQ expression of adherent cells. In adherent cells before preculture, 37.8% of cells were DQ positive. After 4-day culture without rIFN- γ , the proportion of DQ-

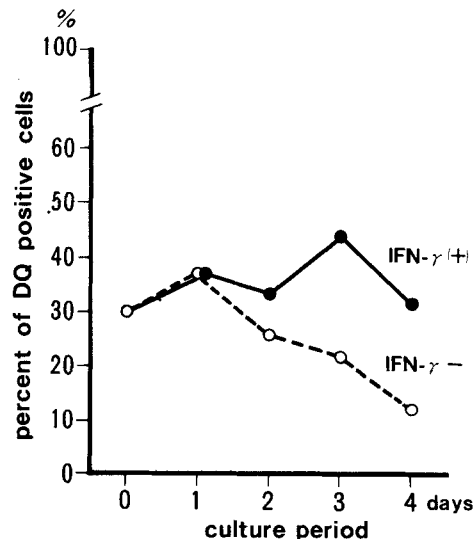


Fig. 3. Decreased DQ expression of adherent cells on culture and conservation by IFN- γ treatment.

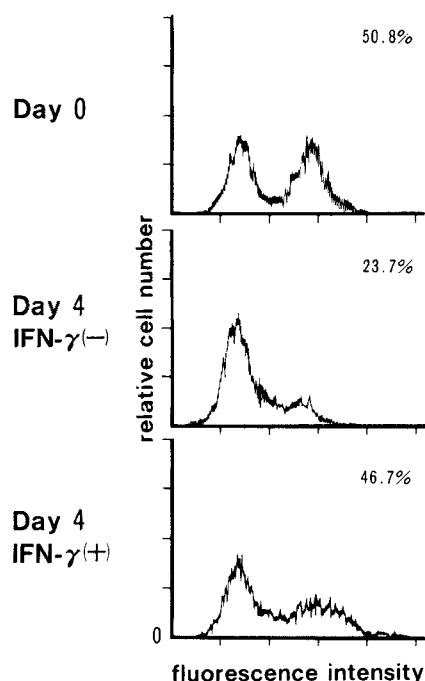


Fig. 4. HLA-DR expression had decreased by 4 days of culture, but improved on addition of INF- γ .

positive cells decreased to 18.1%. Kinetics studies showed that this decrease of DQ expression of adherent cells was concomitant with their decreased IL-2 responsiveness during preculture (Figs 1, 3). This reduced DQ expression was restored to 38.1% by rIFN- γ . Notably, the fluorescence intensity of DQ antigen on rIFN- γ -stimulated cells was markedly enhanced compared with that of uncultured cells. Fig. 3 shows the chronological changes in DQ expression of adherent cells. As is clearly shown, DQ expression of adherent cells was sustained by rIFN- γ . Similarly, the FACS pattern of DR expression of adherent cells was examined (Fig. 4). In uncultured adherent cells, 50.7% were DR positive. After 4-day culture, the proportion of DR-positive cells decreased to 23.7%, but 46.7% of adherent cells cultured with rIFN- γ were DR positive. Fig. 5 shows the chronological changes in DR expression of cultured adherent cells. In the same manner, rIFN- γ corrected the diminution of DR expression on adherent cells during preculture.

Characterization of antigen-presenting adherent cells involved in induction of IL-2 responsiveness

To evaluate the cell type of antigen presenting cells involved in the induction of IL-2 responsiveness, precultured non-T cells were stained by two-color analysis. The cells were stained either with anti-CD14 monoclonal antibody, which recognized CD14 antigen on monocytes, or with anti-CD20 monoclonal antibody, which recognized CD20 an-

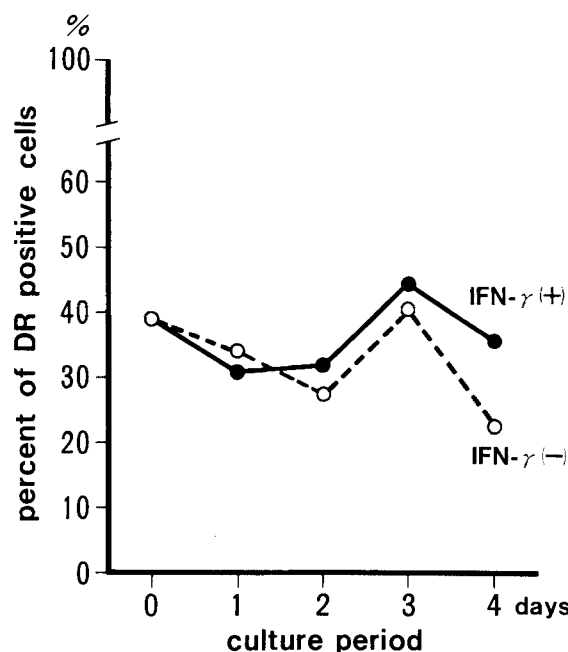


Fig. 5. DR expression of cultured adherent cells and effect of IFN- γ .

tigen on B cells, together with anti-HLA-Class 2 monoclonal antibody. The results indicated that rIFN- γ preferentially improved expression of Class 2 antigen on CD14 positive cells (Table 2).

Discussion

In this study, we report how the antigen-presenting ability of adherent cells decreased when the preincubation time was prolonged without antigen. It was also found that antigen-presenting ability was restored by treatment with rIFN- γ . The antigen-presenting process is composed of uptake, pro-

Table 2. Interferon- γ is capable of maintaining expression of HLA-Class 2 antigen on CD14⁺ more than on CD20⁺ cells

Cell surface markers	Treatment with interferon- γ	Expression of surface antigens	
		Class 2 antigens	Percent of positive cells
CD14 ⁺	-	DP	3.00
	+		7.56
	-	DQ	0.86
	+		3.32
	-	DR	11.96
CD20 ⁺	+		15.98
	-	DP	15.22
	+		19.36
	-	DQ	2.72
	+		3.56
	-	DR	14.60
	+		18.36

cessing and presentation of antigen in the context of appropriate MHC molecules. Expression of Ia antigen on macrophages is diminished after prolonged *in vitro* culture (10). In contrast, increase of expression of Ia antigen is observed after incubation of macrophages in media containing IFN- γ (11, 12). The modulation of Ia expression on macrophages correlates with their ability to present antigen to MHC-restricted proliferation of helper T cell clones (12). We therefore investigated the relation between the improvement in antigen-presenting capacity of adherent cells and the HLA Class 2 expression on these cells. The expression of HLA-DQ molecules on adherent cells decreased after prolonged *in vitro* culture, concomitantly with the reduction of their ability to induce IL-2 responsiveness. The treatment of adherent cells with rIFN- γ corrected the diminution of the HLA-DQ expression and of the induction of IL-2 responsiveness at the same time. Expression of HLA-DR also decreased after prolonged *in vitro* culture and was improved by treatment with rIFN- γ . However, the change of HLA-DQ expression was more closely related with the change of induction of IL-2 responsiveness than that of HLA-DR expression. After 4-day culture HLA-DQ and HLA-DR positive cells were reduced to 24% and 18% of adherent cells respectively, with a concomitant reduction in the density of HLA-DQ and -DR antigens. It seems possible that the antigen presentation is actually carried out by a minority of adherent cells and that INF- γ up-regulates class II antigen on these minority cells. The involvement of HLA-DQ but not of HLA-DR antigen in the induction of IL-2 responsiveness is further supported by the finding that monoclonal antibody to HLA-DQ framework (Leu 10 and clonab DQ), but not to HLA-DR (OKIa1) or HLA-DP (HLA-DP and clonab DP-DR), blocked the ability of the antigen-presenting cells to induce IL-2 responsiveness (5). The present data reconfirmed that DQ-bearing adherent cells from allergic donors played a key role in presenting Df antigen to allergen-specific responder T cells, which were very likely to be members of the CD4 positive subset (5). Most importantly, these HLA-DQ antigens seemed functionally related to the antigen presentation in the induction of Df-specific IL-2 responsiveness.

IL-2 responding cells activated by allergen may function as helper cells for the generation of IgE or as effectors of delayed-type hypersensitivity reactions in allergic children. INF- γ has been reported to be produced by Type 1 helper T cells (T_H1) like cells (13) and to activate the effector macrophages antigen-specifically. In contrast INF- γ suppresses both the production of interleukin 4 (IL-4) from

Type 2 helper T cells (T_H2) like cells and that of IgE from B cells (14). Thus INF- γ may play an important role in these antigen-specific reactions.

The up-regulating mechanism of IFN- γ in HLA Class 2 expression is not fully understood. Receptors for IFN- γ have been reported to be found on several kinds of cells (15–18). It was also reported recently that adherent monocytes had low affinity receptors which were disulfide-linked and differed structurally from the high affinity receptors on nonadherent monocytes (19). However, little is known about the signal transduction mechanism responsible for IFN- γ -inducible Class 2 molecule expression, except for the study by Nezu et al. in which IFN- γ induced this expression via a PKC pathway (20).

In contradiction to this idea, when human-IFN- γ was microinjected into murine macrophages, a time and dose-dependent induction of Ia was detected by autoradiography on the surface of injected and neighbouring cells. This effect on Ia gene expression induced by microinjected human-IFN- γ was in part indirect: granulocyte/macrophage-CSF (GM-CSF) was released by IFN- γ -injected macrophages, and this secondary mediator appeared to induce Ia on neighbouring cells, inasmuch as anti-GM-CSF blocked Ia induction. Anti GM-CSF antibody also partially blocked Ia induction by extracellular murine IFN- γ on murine macrophages. Thus, at least some of the Ia induction attributed to IFN- γ was mediated by GM-CSF (21). Moreover, on human monocytic cell lines (U937, HL60) and peripheral blood monocytes, IFN- γ -binding capacity was down-regulated upon incubation with GM-CSF at a post-translational level, and this down-regulation was caused by a decrease in the number of IFN- γ receptors rather than by a change in affinity (22). In our study, the optimal concentration of IFN- γ was 125 U/ml, but dose-dependent effects were not obtained, which may be due to this down-regulation of IFN- γ receptors by GM-CSF.

We also evaluated the cell type of antigen-presenting cells involved in the induction of IL-2 responsiveness. Pre-cultured adherent cells were stained by two-color analysis. They were stained with anti-CD14 monoclonal antibody, which exclusively recognized monocytes, or with anti-CD20 monoclonal antibody which identified most B cells. IFN- γ improved the expression of the HLA-class II molecule more on CD14-positive than on CD20-positive cells. It has been reported that all peripheral blood monocytes have the capacity to become HLA-DQ and HLA-DP positive; that IFN- γ up-regulates expression of all three loci of human Ia on macrophages; and that IFN- γ does not significantly modulate Ia expression on B cells

(23). These results indicate that HLA-DQ antigen on monocytes plays a major role in presenting antigen to CD4 T cells during Df-specific induction of IL-2 responsiveness.

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