

Interleukin-1 favours the *in vitro* development of type 2 T helper (Th2) human T-cell clones

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SUMMARY

The effects exerted by interleukin-1 (IL1) on the growth and differentiation of human Th1 and Th2 cells were examined. Neither IL1 nor the IL1 receptor antagonist (IL1ra) had detectable activity toward the antigen- or anti-CD3 antibody-induced proliferative response of already established type 1 T helper (Th1) or type 2 T helper (Th2) clones. Moreover, neither exogenous IL1 addition to, nor neutralization of, endogenously produced IL1 in bulk cultures before cloning changed the Th1-like cytokine profile of PPD-specific T-cell lines. Likewise, IL1 addition in bulk culture before cloning did not significantly affect the Th2-like cytokine profile of *Der.p.I*-specific T-cell lines (*Dermatophagoides pteronyssinus* group I). However, *Der.p.I*-specific T-cell lines, derived in the presence of anti-IL1 Ab, IL1ra or the M-20 IL1 inhibitor, exhibited the reduced ability to produce IL4 and an increased ability to produce interferon gamma (IFN γ). More importantly, *Der.p.I*-specific T-cell lines derived in the presence of IL1ra developed into *Der.p.I*-specific CD4⁺ T-cell clones showing a Th0/Th1-like, instead of a Th0/Th2-like, cytokine profile. These data suggest that IL1 is not required for the growth of already established human Th1 or Th2 CD4⁺ T-cell clones and has no regulatory effects on the *in vitro* development of Th1-like cells, but it plays a critical role in the development of Th2-like cells.

Key-words: IL1, T lymphocyte, Interleukin; Th1, Th2, IL1-receptor antagonist, CD4, IL4, IL5, IFN γ .

INTRODUCTION

The initial observation by Mosmann *et al.* (1986) that established murine T helper (Th)

clones could be segregated according to their distinctive cytokine profiles has been amply confirmed in the human system (Wierenga *et al.*, 1990; Parronchi *et al.*, 1991; Del Prete *et al.*,

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1991; Haanen *et al.*, 1991; Yssel *et al.*, 1991; Salgame *et al.*, 1991). In general, stimulation of human CD4⁺ Th lymphocytes *in vitro* with intracellular bacteria antigens results in the development of a restricted Th1-like pattern of cytokine production, *i.e.* IL2, IFN γ and tumour necrosis factor- β (TNF β), but not IL4 and IL5 (Del Prete *et al.*, 1991; Haanen *et al.*, 1991; Yssel *et al.*, 1991; Salgame *et al.*, 1991), whereas stimulation with helminth components or allergens results in the development of CD4⁺ T-cell lines and clones showing a Th2-like profile (production of IL4 and IL5 but not of IFN γ) (Wierenga *et al.*, 1990; Parronchi *et al.*, 1991; Del Prete *et al.*, 1991). A number of clones with an intermediate cytokine profile (Th0) have also been described (Parronchi *et al.*, 1991; Maggi *et al.*, 1988; Paliard *et al.*, 1990). In both murine and human systems, Th1 and Th2 cells may differentiate from a common pool of precursors (Swain *et al.*, 1988; Romagnani *et al.*, 1992) along differentiation pathways controlled at least in part by cytokines produced by lymphocytes and accessory cells. Recent work has focused attention on a number of monokines produced by antigen-presenting cells (APC) capable of influencing the subsequent development of a Th1 or Th2 response. Thus IFN α , IL12 and TGF β have been found to preferentially induce Th1 expansion in various systems (Romagnani, 1992; Swain *et al.*, 1991), whereas IL10 was implicated in Th2 expansion when spleen cells, as opposed to B cells, were used as APC (Hsieh *et al.*, 1992).

In this study, we analysed whether IL1 can affect the *in vitro* differentiation of Th-cell subsets. We show that neither addition nor neutralization of IL1 was able to influence the growth of already established Th1 or Th2 clones. In ad-

dition, IL1 apparently did not exert any significant effect on the development of PPD-specific T cells into Th1 clones. In contrast, neutralization of IL1 in bulk cultures during *in vitro* stimulation with *Der.p.I* shifted the differentiation of *Der.p.I*-specific T cells from the Th0/Th2 to the Th0/Th1 profile. Thus, IL1 and/or IL1ra produced by APC might play an important role in regulating the development of Th2-like responses.

MATERIALS AND METHODS

Donors

Peripheral blood mononuclear cells (PBMC) were obtained from healthy volunteers showing delayed-type hypersensitivity skin reactions to PPD (purified protein derivative), and from atopic house dust mite-sensitive subjects suffering from atopic dermatitis and/or extrinsic asthma and showing immediate type skin reactivity to *Der.p.I*.

Reagents

PPD was a gift of Istituto Sieroterapico e Vaccinogeno Sclavo (Siena, Italy). *Der.p.I* was a gift of Lofarma Allergeni (Milan, Italy). Phytohaemagglutinin (PHA) was purchased from Gibco Laboratories (Grand Island, NY). Anti-CD3 monoclonal antibody (mAb) was purchased from Ortho Pharmaceuticals (Raritan, NJ). Anti-CD4 and anti-CD8 mAb were purchased from Becton-Dickinson & Co. (Mountain View, CA). Human recombinant IL2 was a gift of Eurocetus (Milan, Italy). Human recombinant IL1 α was a gift of Dainippon Pharmaceutical Co. Ltd (Osaka, Japan). Rabbit anti-human IL1 α Ab was purchased from Genzyme (Cambridge, MA). Human recombinant IL1ra has already been described (Arend *et al.*, 1990; Dinarello, 1991). The M-20 IL1 inhibitor (Barak *et al.*, 1986, 1991) was prepared and purified as reported elsewhere (Peritt *et al.*, 1992; Peled *et al.*, 1992).

Ab	= antibody.
APC	= antigen-presenting cell.
cpm	= counts per minute.
<i>Der.p.I</i>	= <i>Dermatophagoides pteronyssinus</i> group I.
FCS	= foetal calf serum.
IFN	= interferon.
IL	= interleukin.
mAb	= monoclonal antibody.
MHC	= major histocompatibility complex.

NK	= natural killer.
PBMC	= peripheral blood mononuclear cell.
PHA	= phytohaemagglutinin.
PPD	= purified protein derivative.
ra	= receptor antagonist.
TGF	= transforming growth factor.
Th	= T helper.
Th0	= type 0 Th.
TNF	= tumour necrosis factor.

Generation of antigen-specific T-cell lines and T-cell clones

PPD- and *Der.p.I*-specific T-cell lines were generated according to a technique previously described (Parronchi *et al.*, 1991; Del Prete *et al.*, 1991). Briefly, 10^6 PBMC in 2 ml RPMI-1640 medium supplemented with 2 mM L-glutamine, 2×10^{-5} M 2-mercapto-ethanol and 5 % human serum (complete medium) were stimulated in 24-well flat-bottomed plates for 5 days with *Der.p.I* (10 μ g/ml) in the absence or in the presence of IL1 (200 U/ml), IL1ra (100 ng/ml), anti-IL1 α Ab (5 μ g/ml) or the M-20 IL1 inhibitor (25 % vol/vol) or with PPD (1 μ g/ml) in the absence or in the presence of IL1 (200 U/ml) or IL1ra (100 ng/ml), respectively. Human IL2 (20 U/ml) was then added and cultures continued for an additional 9 days. Viable T blasts were resuspended in complete medium and tested for their antigen specificity prior to the cloning procedure. To assess the antigen specificity of T-cell lines, 2×10^4 T blasts were seeded in microplates and cocultured for 48 h with irradiated (6,000 rad) autologous PBMC (1×10^5) in the presence of medium alone, *Der.p.I* (1 μ g/ml) or PPD (1 μ g/ml), respectively. After a 16-h pulse with 0.5 μ Ci ^{3} H-thymidine (Amersham International; Amersham, UK), cultures were harvested and radioactivity measured by liquid scintillation. To generate T-cell clones, T blasts obtained from antigen-specific T-cell lines were seeded under limiting dilution conditions (0.3 cell/well) in 96-microwell round-bottomed plates containing 10^5 irradiated (6,000 rad) allogeneic PBMC cells (as feeder cells) and PHA (1 % vol/vol) in a final volume of 0.2 ml complete medium supplemented with IL2 (20 U/ml) and 10 % FCS (Hyclone Laboratories Inc.; Logan, UT), as reported elsewhere (Haanen *et al.*, 1991; Arend *et al.*, 1990; Dinarello, 1991). Growing microcultures were then supplemented, at weekly intervals, with IL2 (20 U/ml) and 10^5 irradiated feeder cells. The phenotype of T blasts from T-cell lines and T-cell clones was examined by flow cytometry and their antigen specificity assessed by measuring ^{3} H-thymidine uptake after 60 h stimulation with the appropriate antigen under MHC-restricted conditions (Parronchi *et al.*, 1991; Del Prete *et al.*, 1991). When the stimulation index (ratio between the mean counts per minute obtained in cultures stimulated with APC plus antigen and the mean cpm obtained in cultures with APC alone) was > 10 , responses were considered as positive. Clonal efficiency was evaluated according to Taswell (1981).

Induction of cytokine production by antigen-specific T-cell lines and T-cell clones

To induce cytokine production, T blasts were resuspended at a concentration of 10^6 /ml in com-

plete medium and stimulated for 24 h with anti-CD3 mAb coated to plate wells according to the method described by Thoma and Lipsky (1991). Cell-free culture supernatants were collected, filtered and stored in aliquots at -70° until used.

Quantitation of IFN γ , IL4 and IL5

The quantitative determinations of IFN γ and IL4 were performed by a commercial RIA (Centor Inc., Malvern, PA) and ELISA (Quantikine R & D Systems, Minneapolis), respectively. The quantitation of IL5 was performed by using the murine LyH7.B13 cell line as indicator cell, as described (Parronchi *et al.*, 1991; Del Prete *et al.*, 1991). Levels of cytokine ± 3 SD over those of control supernatants obtained by stimulation of irradiated feeder cells alone were regarded as positive.

RESULTS

In a first series of experiments, the effect of IL1 or IL1ra on the antigen- or anti-CD3 Ab-induced proliferative response of already established Th1 and Th2 clones was assessed. Neither IL1 addition nor IL1 neutralization had a significant potentiating or inhibitory effect on the proliferation of either the Th1 or Th2 clone (data not shown). The possibility that addition or neutralization of IL1 in bulk culture before cloning could influence the development of antigen-specific CD4 $^+$ T cells into the Th1 or Th2 phenotype was subsequently investigated. To do this, PBMC from 3 normal individuals were stimulated with PPD in the absence or in the presence of different concentrations of IL1 or IL1ra. Likewise, PBMC from 4 atopic house dust mite-sensitive donors were stimulated with *Der.p.I* in the absence or in the presence of different concentrations of IL1 or IL1ra. The resulting T-cell lines were then assessed for their profile of cytokine production in response to insolubilized anti-CD3 Ab. IL1(–) and IL1(+), as well as IL1ra(–) and IL1ra(+) PPD-specific cell lines showed comparable cytokine profiles. Likewise, no differences in the cytokine profile between IL1(–) and IL1(+) *Der.p.I*-specific T-cell lines were observed (data not shown). In contrast, IL1ra(+) *Der.p.I*-specific T-cell lines produced significantly higher concentrations of

IFN γ and significantly lower concentrations of IL4 than IL1ra(-) T-cell lines (fig. 1). The modulatory effect of IL1ra on the development of *Der.p.I*-specific T-cell lines was really the result of IL1 removal from bulk cultures. Indeed, in three subsequent experiments, the effects observed by adding to bulk culture either anti-IL1 Ab or the M-20 IL1 inhibitor (25 % vol/vol) were quite comparable to those exerted by IL1ra (table I).

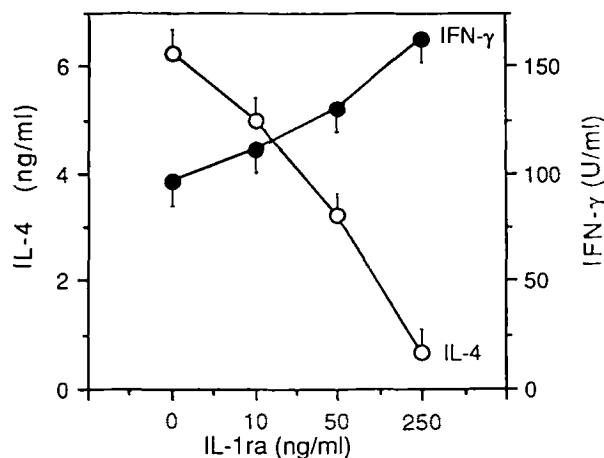


Fig. 1. Production of IL4 (○) and IFN γ (●) by *Der.p.I*-specific T-cell lines derived from PBMC cultured with *Der.p.I* in the absence or presence of IL1ra.

T blasts (10^6 /ml) were stimulated for 24 h with insolubilized anti-CD3 antibody and supernatants were assessed for IL4 and IFN γ content, as described in "Materials and Methods". Mean values $\pm$ SE ($n=4$) are reported.

We finally asked whether changes induced in the cytokine profile of *Der.p.I*-specific T-cell lines by IL1 neutralization in bulk cultures really reflected a shift in the differentiation of *Der.p.I*-specific CD4 $^+$ T cells toward the Th1-like profile. To this end, T blasts from the IL1ra(-) and IL1ra(+) T-cell lines derived from one donor were cloned. Eleven IL1ra(-) and 13 IL1ra(+) *Der.p.I*-specific CD4 $^+$ T-cell clones were obtained and assessed for their ability to produce IFN γ and IL4 in response to stimulation with anti-CD3 mAb. As shown in figure 2, the proportion of IL1ra(-) T-cell clones able to produce IL4 (11/11) was significantly higher than that (7/13) of IL1ra(+) clones ($p < 0.01$). Likewise, the mean IL4 concentration produced by IL1ra(-) clones (1.5 ± 0.4 ng/ml) was significantly higher than that (0.4 ± 0.2 ng/ml) of IL1ra(+) clones ($p < 0.05$). In contrast, the mean concentration of IFN γ produced by IL1ra(-) clones (142 ± 62 U/ml) was significantly lower than that (368 ± 84 U/ml) of IL1ra(+) clones ($p < 0.05$).

DISCUSSION

Studies in mouse with established cloned CD4 $^+$ lines have demonstrated that Th1 and Th2 clones differ in their proliferative responses to antigens presented by different APC. In particular, Th2 clones proliferate weakly or not at all when stimulated with purified B cells, unless

Table I. Production of IL4, IL5 and IFN γ in response to stimulation with insolubilized anti-CD3 mAb by *Der.p.I*-specific T-cell lines derived in the absence or the presence of IL1ra, anti-IL1 or the M-20 IL1 inhibitor.

Bulk culture conditions (*)	Cytokine production by T cell lines (**)		
	IL4 (ng/ml)	IL5 (U/ml)	IFN γ (U/ml)
<i>Der.p.I</i>	2.2 ± 0.7	2.5 ± 0.4	106 ± 21
<i>Der.p.I</i> + IL1ra	0.2 ± 0.1	< 0.4	183 ± 22
<i>Der.p.I</i> + anti-IL1 Ab	1.3 ± 0.4	0.9 ± 0.3	154 ± 19
<i>Der.p.I</i> + M-20 IL1 inhibitor	1.5 ± 0.6	n.t.	261 ± 32

(*) PBMC (10^6 /ml) were cultured for 5 days with *Der.p.I* ($10 \mu\text{g/ml}$) in the absence or presence of IL1ra (100 ng/ml), anti-IL1 Ab ($5 \mu\text{g/ml}$) or M-20 IL1 inhibitor (25 % v/v), and for 9 subsequent days with IL2 (20 U/ml).

(**) T blasts from *Der.p.I*-specific T-cell lines (10^6 /ml) were stimulated for 24 h with insolubilized anti-CD3 mAb and cytokines produced in the supernatants were measured. Results represent the mean values ($\pm$ SE) obtained in three separate experiments.

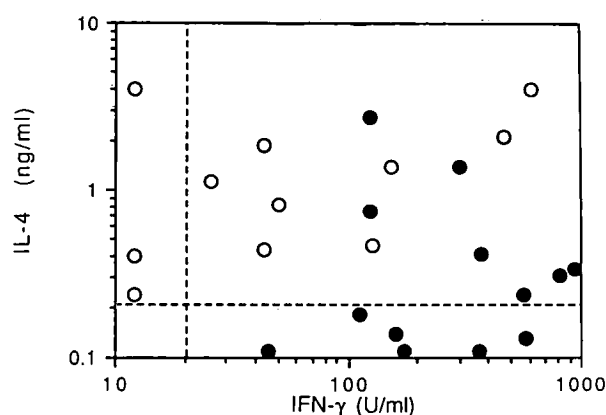


Fig. 2. Production of IL4 and IFN γ by *Der.p.I*-specific CD4 $^{+}$ T-cell clones derived from PBMC cultured with *Der.p.I* in the absence (○) or presence (●) of IL1ra.

Clonal T blasts (10^6 /ml) were stimulated for 24 h with insolubilized anti-CD3 antibody and supernatants were assessed for IL4 and IFN γ content, as described in "Materials and Methods". Dotted lines represent the mean values plus 3 SD of cytokine concentrations produced by irradiated feeder cells alone in response to insolubilized anti-CD3 antibody.

IL1 is added (Williams *et al.*, 1991). This finding together with the demonstration that a competitive antagonist of IL1 blocks the proliferative response of Th2 clones only, support the idea that IL1 is a costimulator for the proliferation of Th2 but not of Th1 cells (Williams *et al.*, 1991). The results of the present studies suggest that IL1 is apparently not required either for the proliferative response of already established human Th1 and Th2 cells, or for the *in vitro* differentiation of PPD-specific T lymphocytes into cells showing a Th1-like profile of cytokine production. However, IL1 neutralization in bulk culture during *in vitro* stimulation with *Der.p.I* favoured the development of human *Der.p.I*-specific T-cell lines and clones producing high concentrations of IFN γ and no or low concentrations of IL4, unlike the T-cell lines and clones generated in the absence of IL1 blockers or competitive antagonists, which usually produce high amounts of IL4 and no or low IFN γ concentrations (Wierenga *et al.*, 1990; Parronchi *et al.*, 1991). In this regard, it is of note that IL1ra was very efficient in reducing the development of IL4-producing cells, whereas the M-20 IL1 in-

hibitor apparently showed higher activity on the development of IFN γ -producing cells. Whether or not this is due to differences in the inhibitory pathway of IL1 activity between the two reagents remains to be established. On the other hand, the M-20 IL1 inhibitor, which is a product of a myelomonocytic leukaemia cell line, is not purified to homogeneity (Barak *et al.*, 1986). Thus, it cannot be excluded that its partially different activity is related to the presence in the preparation of some contaminating polypeptide. Taken together, these data strongly suggest that the presence of even minimal IL1 concentrations during *in vitro* stimulation with antigen are critical for the development of IL4-producing (Th2-like) human T-cell clones. The lack of effect by additive exogenous IL1 probably reflects the fact that, during PBMC activation, IL1 is naturally produced in large excess over IL1ra.

In the last few years, it has become clear that cytokines not only determine the functional phenotype of effector lymphocytes, but also strongly affect their differentiation pathways. IFN γ promotes the differentiation of CD4 $^{+}$ T cells into Th1, both *in vitro* (Gajewsky *et al.*, 1989; Maggi *et al.*, 1992) and *in vivo* (Seder *et al.*, 1992; Scott *et al.*, 1988). Moreover, a number of monokines produced by APC may influence the subsequent development of Th1 responses. Thus, IFN α and IL12, that are produced by macrophages and B cells and induce IFN γ production by natural killer cells, have been found to have important effects in favouring Th1 expansion (Parronchi *et al.*, 1992; Romagnani, 1993; Manetti *et al.*, 1993; Hsieh *et al.*, 1993). Most striking is the requirement for IL4 for maturation of Th2 cells. In numerous systems, IL4 remains the most dominant factor in determining the likelihood for Th2 polarization in cultured cells (Swain *et al.*, 1991; Seder *et al.*, 1992). Production of IL4 is restricted to mature Th2 cells (Mosmann *et al.*, 1986; Wierenga *et al.*, 1990; Parronchi *et al.*, 1991; Del Prete *et al.*, 1991), a small population of early thymic emigrants (Bendelac and Schwartz, 1991) and cells of the mast cell/basophil lineage (Piccini *et al.*, 1991; Bradding *et al.*, 1991; Brunner *et al.*, 1993). Whether one of these populations or a hitherto undescribed cell serves

as the source of IL4 that mediates the subsequent development of Th2 cells, remains unclear. Alternatively, a cytokine that induces IL4 release from these cells, akin to the role of IL12 in inducing IFN γ release from NK cells, may yet be discovered. The results reported here showing that IL1 exerts effects apparently contrary to those of IFN α and IL12 suggest that a fine balance among cytokines produced by APC at the time of antigen presentation may be critical for the regulation of Th-cell development. The possible role of monokine antagonists in this process cannot be excluded. Of note, it has recently been shown that depletion of IL1ra increases egg granuloma formation in *Schistosoma mansoni*-infected animals and shifts the secondary response from a Th1- to Th2-like profile (Chensue *et al.*, 1993), suggesting that also *in vivo* as well IL1 plays a critical role in the development of Th2 cells. Whether IL1 favours early IL4 production from a particular T- or non-T-cell subset, or whether it is acting in concert with IL4 in favouring Th2-cell development remains to be established.

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L'interleukine-1 favorise le développement *in vitro* de clones Th2 humains

Les effets exercés par l'interleukine-1 (IL1) sur la croissance et la différenciation des cellules Th1 et Th2 humaines ont été examinés. Ni l'IL1 ni même l'antagoniste du récepteur de l'IL1 (IL1ra) ne présentent une activité détectable sur la réponse proliférative induite par l'antigène ou par l'anticorps anti-CD3 sur des clones T auxiliaires de type 1 (Th1) ou de type 2 (Th2) déjà stabilisés. De plus, ni l'addition d'IL1 exogène ni la neutralisation de l'IL1 produite de façon endogène, dans des cultures avant le clonage, ne modifient le profil de cytokines de type Th1 des lignées cellulaires spécifiques de la PPD (purified protein derivative). De même, l'ajout d'IL1 dans la culture avant le clonage ne modifie pas de façon signi-

ficative le profil de cytokines de type Th2 des lignées cellulaires T spécifiques de *Dermatophagoides pteronyssinus* groupe I (*Der.p.I*). Cependant, les lignées cellulaires T spécifiques de *Der.p.I*, établies en présence d'anticorps anti-IL1, d'IL1ra ou de l'inhibiteur de l'IL1, M-20, présentent une aptitude réduite à produire de l'IL4 et une aptitude accrue à produire de l'IFN γ . De façon plus intéressante, les lignées cellulaires T spécifiques de *Der.p.I* établies en présence d'IL1ra se développent en clones T CD4⁺ spécifiques de *Der.p.I* présentant un profil de cytokines de type Th0/Th1 au lieu de Th0/Th2. Ces résultats suggèrent que l'IL1 n'est pas nécessaire à la croissance des clones humains Th1 ou Th2 CD4⁺ et n'a pas d'effets régulateurs sur le développement des cellules Th1, mais que l'IL1 joue un rôle important dans le développement des cellules Th2.

Mots-clés: IL1, Lymphocyte T, Interleukine; Th1, Th2, Antagoniste du récepteur de l'IL1, CD4, IL4, IL5, IFN γ .

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