Clin Exp Immunol 1994; 98:151–157

Different growth factor requirements for human Th2 cells may reflect *in vivo* induced anergy

F. C. VAN REIJSEN*, O. L. C. WIJBURG*†, M. GEBHARDT‡, A. G. VAN IEPEREN-VAN DIJK*, S. BETZ§, E.-M. POELLABAUER†, T. THEPEN*, C. A. F. M. BRUIJNZEEL-KOOMEN* & G. C. MUDDE† *Department of Dermatology/Allergology, University Hospital, Utrecht, The Netherlands, †Sandoz Research Institute, Vienna, Austria, ‡Universitäts-Hautklinik, Jena, Germany, and §Alexanderhaus, Davos, Switzerland

(Accepted for publication 24 June 1994)

SUMMARY

We previously reported the isolation of allergen-specific Th2 lines and clones from atopy patch test (APT) sites of atopic dermatitis (AD) patients. Upon stimulation with allergen or anti-CD3+ phorbol myristate acetate (PMA) IL-4 was released with or without IL-5, while no (or extremely low concentrations of) IL-2 and interferon-gamma (IFN-γ) were detectable. A high IL-4/IFN-γ ratio facilitates production of allergen-specific IgE, of which high levels are observed in AD patients. Here, we show that the above mentioned Th2 cells are notably different from murine Th2 cells. Not IL-4, which is the autocrine acting growth factor for murine Th2 cells, but IL-2 was needed for proliferation of these human APT-derived Th2 lines and clones. Of significance, unless exogenous IL-2 was added, no proliferative response to allergen, presented by Epstein–Barr virus-transformed B (EBV-B) cells, non-T cells or IgE-bearing Langerhans cells (LC), occurred. Lack of proliferation and IL-2 production after full T cell receptor (TCR) triggering is a characteristic first described for *in vitro* anergized T cells. However, like the clones we describe in this study, anergic T cells may retain production of cytokines other than IL-2. A further resemblance between anergic T cells and the human Th2 clones reported here is that IL-4 can enhance IL-2-driven proliferation, but is not capable of inducing T cell growth by itself. The absence of IL-4-driven proliferation differentiates human Th2 cells from murine Th2 cells. Both produce IL-4 when stimulated in a cognate fashion, but only murine Th2 cells will proliferate. We conclude that the presently reported human Th2 cells are different from murine Th2 cells, in that they need other T cells to produce IL-2 required for their expansion. Moreover, the Th2 cells phenotypically resemble anergic T cells. As yet, however, we have no clue as to whether these features account for the current Th2 cells only or for human Th2 cells in general. We hypothesize that the Th2 phenotype of AD skin-derived, allergen-specific T cells may be induced *in vivo* by LC, which lack CD80, and therefore do not provide secondary signals through CD28–CD80 interaction.

Keywords Th2 anergy IL-2 IL-4 atopic dermatitis

INTRODUCTION

Patients suffering from atopic dermatitis (AD) are characterized by elevated serum levels of IgE specific for otherwise harmless, environmental allergens like the housedust mite (*Dermatophagoides pteronyssinus* (Dpt)) [1]. In normal subjects, IgE is produced in defence against helminths, and may facilitate antigen presentation by B cells and dendritic cells [2–5]. IgE synthesis in both mouse [6,7] and man [8] is reciprocally regulated by IL-4 and interferon-gamma (IFN-γ), which are, as was first described in mice, secreted by two distinct panels of Th cells. Th1 cells, secreting IL-2 and IFN-γ, induce DTH, whereas Th2 cells, releasing IL-4 and IL-5 upon activation, promote immunoglobulin production by B cells [9]. Recently, evidence was provided for the existence of human Th1 and Th2 cells [10].

Atopic patients show immediate type skin reactions to environmental allergens on intradermal application [11]. Moreover, in AD patients exclusively, eczematous skin lesions can be induced after epicutaneous application of these allergens to the skin. This atopy patch test (APT) reaction reaches a maximum intensity 24–48 h after testing. The histopathology of the APT resembles lesional skin with respect to the infiltration of dendritic cells and T cells [12]. The T cell infiltrate from both APT sites [13,14] and lesional skin [15,16] contains allergen-specific Th2 cells.

We recently reported the isolation of Dpt-specific T cell

Correspondence: Frank C. van Reijsen, University Hospital Utrecht, Department of Dermatology/Allergology, Postbus 85500, NL-3508 GA Utrecht, The Netherlands.

lines and clones from APT sites of AD patients [13]. The cultures were mainly of Th2 phenotype: after stimulation with allergen or anti-CD3+ phorbol myristate acetate (PMA), high amounts of IL-4 were secreted, while no IFN-γ and IL-2 were detected. IL-2, but not IL-4, facilitated proliferation of these Th2 cultures. Moreover, since no (detectable) IL-2 was released, IL-2 had to be added for allergen-specific proliferation. In the present study we extend these observations by showing that IL-4, although being impotent by itself, can powerfully enhance IL-2-driven proliferation. The above mentioned characteristics, namely biased cytokine production, lack of proliferation and IL-4-mediated amplification of IL-2-driven proliferation, are shared with anergic T cells [17–19]. The following study deals with the question, whether human Th2 cells are in fact anergic T cells, or should be considered a subpopulation of T cells which, in contrast to T cells described so far, do not secrete their own (autocrine) growth factor, but are dependent on paracrine acting IL-2 from other T cells.

MATERIALS AND METHODS

Reagents

Culture media were based on RPMI 1640 supplemented with $NaHCO_3$ (2 mg/ml) (Seromed, Biochrom KG, Berlin, Germany), penicillin (100 U/ml), streptomycin (100 μg/ml) and glutamine (2 mM) (GIBCO, Grand Island, NY). CM-ABS contained 10% pooled human, blood group AB, rhesus positive serum (ABS), whereas CM-FCS contained 10% fetal calf serum (FCS; GIBCO). Human rIL-2 and rIL-4 were kind gifts from Dr E. Liehl (Sandoz Forschungsinstitut, Vienna, Austria); IL-2 and IL-4 were both used at concentrations of 50 U/ml unless otherwise stated. Goat anti-mouse (GaM) IgG was obtained from Cappel (Organon Teknika Corp., West Chester, PA) and Leu-4 (anti-CD3) from Becton Dickinson (Mountain View, CA). Freeze-dried Dpt and grass pollen allergen (GP; mixture II, containing *Agrostis stolonifera*, *Anthoxanthum odoratum*, *Dactylis glomerata*, *Lolium perenne*, *Arrhenatherum elatius*, *Festuca rubra*, *Poa pratensis*, *Secale cereale*, *Holcus lanatus* and *Phleum pratense*) were kind gifts from Haarlems Allergenen Laboratorium (Haarlem, The Netherlands). Calcium ionophore A23187 (CI), PMA and phytohaemagglutinin (PHA) were purchased from Sigma (St Louis, MO).

Patients

Two adult patients (CF4 and CF7) with AD (diagnosis in accordance with the criteria of Hanifin & Rajka [20]) were included in this study, after giving their informed consent. Clinically non-involved skin (from the upper part of the back) of patient CF4 was challenged epicutaneously (APT) [21] with Dpt (10 000 AU/ml). APT sites were read 12, 24 and 36 h after allergen application, and considered positive when erythema and papules were present. Patient CF7 was only intradermally tested (IT) with GP (100 AU/ml). The skin reaction was read after 12 h and 24 h. Test sites were cleaned with alcohol, after which 3 mm punch biopsies were taken (using 1% lidocain as a local anaesthetic). Patient CF4 had a punch biopsy taken from lesional skin in addition. The patients did not use systemic corticosteroids or antihistamines. Local corticosteroid therapy was stopped at least 1 week before patch APT.

T cell cloning procedure

T cells were cloned as described before [13]. Briefly, biopsies were minced with two scalpels in CM-ABS; no proteolytic enzymes were used. Skin fractions from each biopsy were cultured in a 96-well flat-bottomed plate in CM-ABS containing IL-2 + IL-4 (both 50 U/ml). After 21 days, cells from responding wells were counted by 0·1% trypan blue exclusion. Viable cells were subcloned in 96-well U-bottomed plates under limiting dilution conditions with allogeneic, irradiated (40 Gy) Epstein–Barr virus-transformed B (EBV-B) cells (10^4 per well) [22] in CM-ABS supplemented with IL-2 + IL-4. No mitogens were used in the limiting dilution cultures. Cells from responding wells were expanded by restimulation with immobilized anti-CD3 and culturing in CM-ABS containing IL-2 + IL-4.

T cell lines and clones of about 10^5 cells were transferred into 24-well plates, expanded and tested for allergen specificity in a lymphocyte stimulation test (LST) and a granulocyte-macrophage colony-stimulating factor (GM-CSF) production assay. All T cell lines and clones presented here were $CD4^+$ and expressed the α/β T cell receptor (TCR) (data not shown).

Lymphocyte stimulation and expansion tests

LST were performed in triplicate in 96-well U-bottomed plates in CM-ABS. Each well contained 4×10^4 resting T cells and antigen-presenting cells (APC) (4×10^4 EBV-B cells, 4×10^4 non-T cells or 10^3 Langerhaus cells (LC) [2]). In interleukin-supplemented stimulations, 50 U/ml of IL-2 and/or 50 U/ml IL-4 were added. Before cocultivation with T cells, the APC were incubated overnight (EBV-B cells) or 1 h (non-T cells and LC) at 37°C with allergen (Dpt: 50 μg/ml for non-T cells and LC and 250 μg/ml for EBV-B cells; GP: 50 μg/ml) in CM-FCS. Non-preincubated APC were prepared as medium control for T cell stimulation. Subsequently, APC were irradiated (40 Gy) and excess allergen was removed by washing twice (250 *g*, 10 min at room temperature). After 4 days 1 μCi ^{3}H-thymidine per well was added and incorporation was measured 16 h later.

Lymphocyte expansion tests (LET) were performed in 24-well plates. Cultures contained 1×10^6 T cells, 1×10^6 Dpt-preincubated EBV-B cells and 50 U/ml IL-2 and/or 50 U/ml IL-4. Control cultures with T cells and non-preincubated EBV-B cells were included. After 14 days viable cells were counted by trypan blue exclusion.

Cytokine release

T cells (10^6) were stimulated with immobilized anti-CD3 in 1 ml CM-ABS supplemented with PMA (10 ng/ml). Stimulation was performed in a 24-well plate and after 24 h cultivation at 37°C, supernatants were collected, filtered (0·22 μm) and stored at −20°C until quantification.

Measurement of cytokines in T cell clone supernatants

The assays used for quantification of IL-2 and IL-5 in supernatants of T cell cultures have been described in detail elsewhere [23,24]. Briefly, IL-2 and IL-5 bio-activities were measured by adding different concentrations (1 : 2 and 1 : 40) to CTLL-2 and B13 murine cell lines as indicator cells, respectively. Semiquantitative estimates of IL-2 and IL-5 produced by each T cell line or clone were obtained by standard curves, using human rIL-2 and mouse rIL-5 [25], respectively.

IFN-γ was determined by ELISA (EASIA ELISA-kit;

Table 1. Cytokine secretion profiles

Clone	IL-2 (U/ml)	IL-4 (pg/ml)	IL-5 (U/ml)	IFN-γ (U/ml)
4:2.1	0	805	4·8	0
4:2.6	0	239	1·8	0
4:2.15	0	13 600	0	0
4:2.24	0	813	0	0
4:2.80	2·3	2 300	1·8	19
4:3.1	0	6 100	1·2	0
4:7.6	0·6	3 270	0·5	9
4:14.4	0	748	0	0

Medgenix Diagnostics, Fleurus, Belgium) according to the manufacturer's recommendations. Here, supernatants were diluted 1:10, resulting in a detection limit of 2·5 U/ml.

GM-CSF and IL-4 were quantified by non-commercial sandwich ELISA, as previously described [13].

RESULTS

IL-2 is the growth factor for APT-derived Th2 clones

The growth factor requirements for proliferation of one 12-h APT (4:7·6), three 24-h APT (4:2·15, 4:2·80 and 4:3·1) and one 36-h APT (4:14·4)-derived Dpt-specific Th2 clone (cytokine secretion profiles are listed in Table 1) were investigated. The clones were challenged in an LST, lasting 5 days, with Dpt, Dpt + IL-2, Dpt + IL-4 and Dpt + IL-2 + IL-4. When no interleukins were supplied, allergen-induced proliferation was absent (Fig. 1a). Addition of IL-4 (Fig. 1c) somewhat amplified the allergen-induced T cell activation in two out of five cases. In contrast, IL-2 showed growth factor activity in all cultures (Fig. 1b) and, moreover, was at least 3·6 times more powerful than IL-4. Only minor elevation of the IL-2-enhanced proliferation was achieved by additional IL-4 in two out of five clones (Fig. 1d).

To exclude that the requirement of exogenous IL-2 for allergen-induced proliferation was inherent to the use of EBV-B cells as APC, four 24-h APT-derived T cell clones were challenged with IgE^+ LC (Fig. 2a), non-T cells (Fig. 2b) and EBV-B cells (Fig. 2c) preincubated with Dpt. The data clearly show that, although non-T cells and LC were mostly better APC than EBV-B cells, none of the three APC types could promote exogenous IL-2-independent proliferation.

Thus, IL-2 and not IL-4 is crucial for proliferation of these Th2 phenotype-expressing clones.

IL-4 enhances growth factor activity of IL-2

The effect of IL-2 and IL-4 alone and also IL-2 + IL-4 on proliferation of the T cell clones was then examined in an LET, lasting 14 days instead of 5. Here, expansion in IL-2 + IL-4, following stimulation with Dpt, resulted in a 2·1–9·6 times increased number of T cells compared with culturing in IL-2 alone. When cultured in IL-4 alone, Dpt-stimulated clones died within 7 days. Indices of T cell proliferation in IL-2 + IL-4 and in IL-2 alone (IL-2 + IL-4/IL-2) are shown in Fig. 3 for both LST and LET. Only in the LET did IL-4 potently enhance IL-2-driven T cell growth promotion.

The supporting effect of IL-4 on IL-2-driven proliferation was also observed during the establishment of polyclonal T cell lines and of T cell clones. One half of the 36-h APT biopsy of patient CF4 was cultured with IL-2 + IL-4, while the other was cultured with IL-2 alone. T cell growth was promoted in both

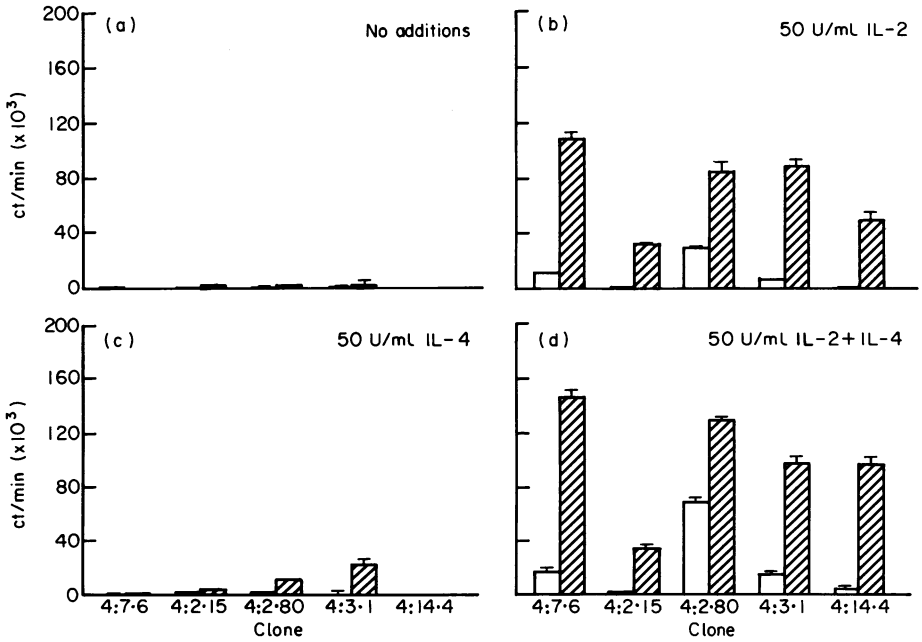

Fig. 1. IL-2 is the growth factor for *Dermatophagoides pteronyssinus* allergen (Dpt)-specific, atopy patch test (APT)-derived Th2 clones. Five clones were screened for Dpt-specific proliferation in a lymphocyte stimulation test (LST) (a) without exogenous interleukins, (b) with IL-2, (c) with IL-4 and (d) with IL-2 + IL-4. T cells stimulated with non-preincubated antigen-presenting cells (APC) (medium) are presented together with T cells stimulated with Dpt-preincubated APC. Data are presented as mean of ct/min ± s.e.m. Ct/min of APC have been subtracted. □, Medium; ▨, Dpt.

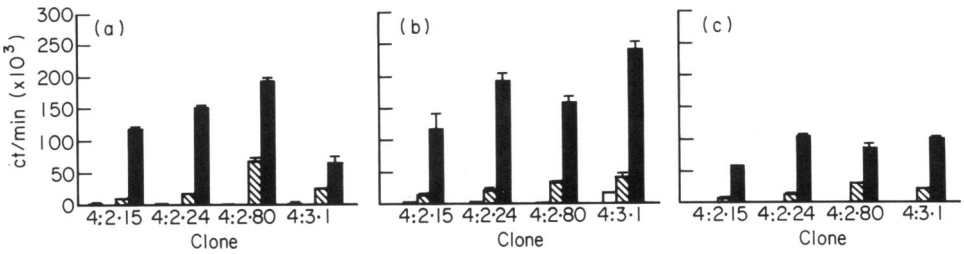

Fig. 2. Lack of proliferation is not caused by the use of Epstein–Barr virus-transformed B (EBV-B) cells as antigen-presenting cells (APC). Four T cell clones were stimulated with *Dermatophagoides pteronyssinus* allergen (Dpt), presented by (a) Langerhans cells (LC), (b) non-T cells and (c) EBV-B cells, with and without IL-2. T cells stimulated with non-preincubated APC (medium) are presented together with T cells stimulated with Dpt-preincubated APC. Data are presented as mean of ct/min ± s.e.m. Ct/min of APC have been subtracted. □, Medium; ▨, Dpt; ▧, medium + IL-2; ■, Dpt + IL-2.

cultures. After 17 days, the cultures were subcloned with and without PHA using limiting dilution (Table 2). No clones were obtained when both IL-4 and PHA were absent (4:10). Addition of PHA to the subcloning (4:10B) and addition of IL-4 throughout the whole procedure (4:14) were equally efficient. PHA in combination with IL-2 and IL-4 resulted in the highest cloning efficiency (4:14B).

These results show that IL-4 enhances the proliferative action of IL-2, which improves *in vitro* T cell expansion and enables PHA-independent T cell cloning.

Impaired IL-2 production and Th2 phenotype are not due to expansion with immobilized anti-CD3 or culturing with IL-4

Our T cell cloning protocol differed from conventional systems. Immobilized anti-CD3 was used instead of PHA as T cell mitogen and IL-4 was introduced as co-growth factor. Therefore, we verified whether these modifications could have induced *in vitro* T cell phenotype alterations. First, in order to substantiate that T cell stimulation with anti-CD3 did not alter growth factor dependency in comparison with PHA, Dpt-specific T cell clones from the 36-h APT expanded with anti-CD3 (4:14) or PHA (4:14B) were stimulated with Dpt in an LST in the presence and absence of IL-2. It is shown in Fig. 4a that T cell clones from both groups were dependent on exogenous IL-2 for proliferation. Second, a 12-h (7B:6) and a 24-h (7B:8) intracutaneous test (IT)-derived T cell line, both isolated in IL-2 alone, were proven IL-2-dependent for allergen (GP) induced proliferation (Fig. 4b). Furthermore, both lines and six T cell clones derived from line 7B:6 showed a Th2 phenotype after anti-CD3 + PMA stimulation (Table 3). These data demonstrate that even when exogenous IL-4 was omitted from the culture medium, lines and clones with a Th2 phenotype and dependent on IL-2 for proliferation could be generated, albeit with reduced efficiency. Finally, by using our T cell cloning method we obtained clones, from a lesional skin biopsy of patient CF4 (4:5), which exhibited diverse cytokine production profiles. Table 4 shows that six T cell clones secreted more than 30 U/ml of IFN-γ, and detectable amounts of IL-2 were released by seven out of 12 clones after anti-CD3 + PMA stimulation. This indicates that stimulation with anti-CD3 and culturing in IL-2 + IL-4 did not necessarily result in isolation of Th2 clones.

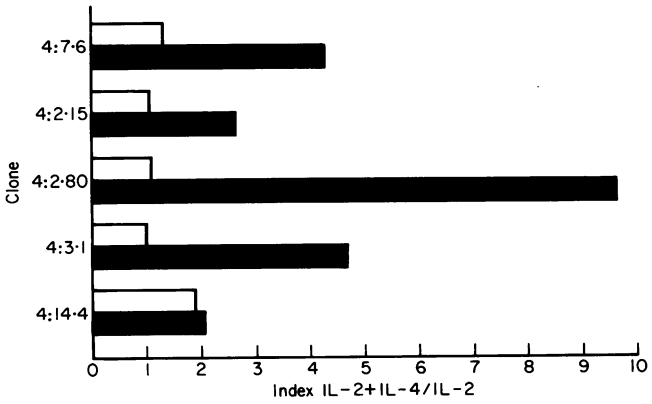

Fig. 3. IL-4 enhances growth factor activity of IL-2. Growth promotion by IL-2 alone and IL-2 + IL-4 were compared when added to *Dermatophagoides pteronyssinus* allergen (Dpt)-stimulated T cell clones in a short term (5 days, lymphocyte stimulation test (LST) (□)) and a long term (14 days, lymphocyte expansion test (LET) (■)) assay. LST and LET data are presented together as IL-2 + IL-4/IL-2 indices.

DISCUSSION

We previously reported T cell lines and clones derived from 24-h APT sites of AD patients, which secreted IL-4 and IL-5 but no IL-2 and IFN-γ upon stimulation with anti-CD3 + PMA as well as allergen [13]. T cell clones with this restricted cytokine production profile were first described in the mouse and classified as Th2 cells [9]. Murine Th2 cells prolife-

Table 2. Subcloning of T cell lines derived from the 36-h atopy patch test (APT) of patient CF4

Line	Isolated in	Subcloned with	Cloning efficiency (%) 0·3 cell/well	Cloning efficiency (%) 1·0 cell/well
4:10	IL-2	IL-2	0	0
4:10B	IL-2	IL-2 + PHA	15·4	11·1
4:14	IL-2 + IL-4	IL-2 + IL-4	14·4	12·2
4:14B	IL-2 + IL-4	IL-2 + IL-4 + PHA	27·7	21·9

PHA, Phytohaemagglutinin.

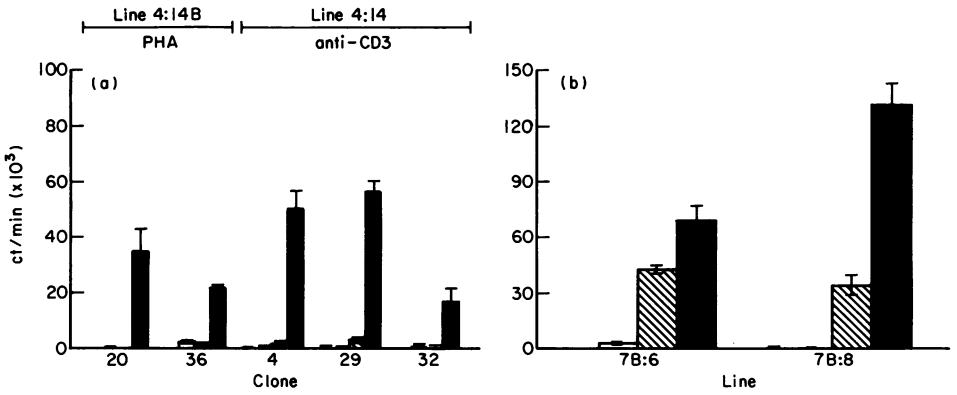

Fig. 4. Use of anti-CD3 and IL-4 in T cell expansion are not responsible for the lack of proliferation. (a) The T cell clones 4:14B.20 and 4:14B.36, subcloned and expanded with phytohaemagglutinin (PHA), and the clones 4:14.4, 4:14.29 and 4:14.32, expanded after mitogen-free subcloning with anti-CD3, were compared with respect to *Dermatophagoides pteronyssinus* allergen (Dpt)-specific proliferation in a lymphocyte stimulation test (LST) with and without IL-2. (b) T cell lines 7B:6 and 7B:8, raised in IL-2 alone, were tested in an LST for grass pollen allergen (GP)-specific proliferation with and without IL-2. T cells stimulated with non-preincubated antigen-presenting cells (APC) (medium) are presented together with T cells stimulated with GP-preincubated APC. Data are presented as mean of ct/min ± s.e.m. Ct/min of APC have been subtracted. (a) □, Medium; ▨, Dpt; ▧, medum + IL-2; ■, Dpt + IL-2. (b) □, Medium; ▨, GP; ▧, medium + IL-2; ■, GP + IL-2.

rate in response to autocrine IL-4 and, although IL-2 can also function as growth factor, can therefore grow independently from other T cells [26,27]. In contrast, human Th2 cells, described by various groups [14,16,23,28], have never been checked adequately for growth factor requirements. These cells were classified according to their cytokine secretion profile only. When we examined the growth factor requirements of our Th2 clones, we observed that they were absolutely dependent on (exogenous) IL-2 for proliferation [13]. Noticeably, concerning this characteristic these human Th2 cells are distinct from classical murine Th2 cells. In fact they closely resemble previously reported murine T cells, which were rendered non-responsive when stimulated via the TCR complex in the absence of costimulatory signals. This state called clonal anergy was further characterized by inhibited IL-2 secretion after subsequent stimulation [29–31].

In the current study we extensively analysed the characteristics of the previously reported clones. Furthermore, in order to exclude that the anergic T cell-resembling features were induced *in vitro*, we compared the growth factor requirements and cytokine production profiles with those of control lines and clones which were obtained under different conditions. In a series of experiments we demonstrate that neither addition of IL-4 to the culture medium nor stimulation with immobilized anti-CD3 caused the aberrant Th2 phenotype. The use of EBV-B cells as APC in LST cannot be considered the reason for IL-2 dependency either, since peripheral blood-derived non-T cells and also skin-derived, IgE^+ LC were incapable of inducing T cell proliferation in the absence of IL-2. Thus, we conclude that the clones which we derived from skin of AD patients possess a functional Th2 phenotype and need paracrine IL-2 from other T cells for *in vivo* proliferation.

Studies from several other investigators show that a Th2 phenotype may indeed be a consequence of clonal anergy. First, stimulation of Th0 clones with fixed APC or other treatments incapable of delivering costimulatory signals inhibited secretion of IL-2 but not IL-4 [19,32]. Second, anergic T cells are not necessarily completely non-responsive. Due to partial activation, effector functions may be exerted in the absence of proliferation [17,33]. By this means, anergic T cells can function as Th2 cells capable of inducing IgE production [13]. Partial

Table 3. Cytokine secretion profiles of T cell lines and clones isolated and cultured in IL-2 alone

Clone/line	IL-2 (U/ml)	IL-4 (pg/ml)	IL-5 (U/ml)	IFN-γ (U/ml)
7B:6	0	320	0	0
7B:8	0	3318	0	0
7B:6.1	0	793	0·8	1
7B:6.2	0	562	0	7
7B:6.3	0	1172	0·3	8
7B:6.5	0	> 3000	0	4
7B:6.8	0	> 3000	0	7
7B:6.9	0	436	0	0

Table 4. Cytokine secretion profiles of lesional skin-derived T cell clones

Clone	IL-2 (U/ml)	IL-4 (pg/ml)	IL-5 (U/ml)	IFN-γ (U/ml)
4:5.5	0·8	208	0	79
4:5.12	0·8	572	0	59
4:5.34	0	522	0	7
4:5.35	0·6	445	0	30
4:5.36	0·4	220	0	74
4:5.40	0·6	633	0	7
4:5.42	2·7	248	1·7	127
4:5.47	7·2	560	0	130
4:5.48	0	0	0	32
4:5.59	0	383	0	10
4:5.70	0	640	0	8
4:5.74	0	363	0	8

activation was even demonstrated *in vivo*, when mice were injected with high doses of TNP-conjugated aqueous antigen. Secretion of IL-2 and IFN-γ, but not IL-4 was reduced [34]. Tolerization in the presence of anti-IL-4 induced elevated levels of IgG2a, IgG2b and IgG3, whereas IgE production was completely abolished. In contrast, injection of anti-IL-4 could not reverse antigen non-responsiveness [35]. Third, in concordance with our data, IL-4 synergized with IL-2 to promote expansion of *in vitro* anergized murine Th0 clones, while it was incapable of inducing T cell growth by itself [19]. In addition, IL-4 was shown to enhance the proliferative effect of IL-2 on primary human AD skin-derived polyclonal T cell cultures [36].

In AD, the skin is an evident site for induction of anergy in allergen-specific T cells. Specific stimulation of peripheral blood-derived T cells by IgE^+ LC strongly correlated with a positive APT reaction to the same allergen in AD patients [2]. In concert with the isolation of allergen-specific T cells from lesional AD skin [15,16] and APT-induced lesions [13,14], this indicates that allergen presentation may take place in the skin. However, freshly isolated epidermal LC do not express CD80 [37] required for delivering costimulatory signals to the T cell via CD28 and CTLA4 [38–40]. As a result epidermal LC may induce anergy in skin-infiltrating, allergen-specific T cells.

Seemingly in contrast with our findings, allergic immune responses have been redirected by means of anergy induction [41]. Supraoptimal concentrations of antigenic peptide abolished IL-4 production in a human Th2 clone, whereas (the reduced) production of IFN-γ was maintained [42]. These data suggest that the conditions under which anergy is induced bias the subsequent T cell phenotype. Thus, the cytokine production of an anergic T cell may be directed towards either IFN-γ or IL-4.

In conclusion, we present evidence that human, allergen-specific, APT-derived Th2 clones are basically different from murine Th2 clones in that they require IL-2 instead of IL-4 for proliferation. The clones share phenotypical similarities with anergic T cells. As yet, it is not clear whether human Th2 cells, dependent on IL-4 for proliferation, exist. In case they do, we must consider the clones discussed here, anergic T cells which unfortunately contribute to the state of allergy. However, if it appears that all human Th2 clones are dependent on IL-2, we will have to reconsider human Th2 cells as a unique T cell population, the expansion of which is controlled by other, IL-2-producing T cells.

ACKNOWLEDGMENTS

We thank Sandoz Research Institute, Vienna, for providing a grant for Frank van Reijsen. Part of the work was supported by the Swiss National Fund.

REFERENCES

1 Juhlin L, Johansson S, Bennich H, Hogman C, Thyresson N. Immunoglobulin E in dermatoses. Arch Dermatol 1969; **100**:12–16.
2 Mudde GC, Van Reijsen FC, Boland GJ, De Gast GC, Bruijnzeel PLB, Bruijnzeel-Koomen CAFM. Allergen presentation by epidermal Langerhans cells from patients with atopic dermatitis is mediated by IgE. Immunology 1990; **69**:335–41.
3 Mudde GC, Hansel TT, Van Reijsen FC, Osterhoff BF, Bruijnzeel-Koomen CAFM. IgE: an immunoglobulin specialized in antigen capture? Immunol Today 1990; **11**:440–3.
4 Santamaria LF, Bheekha R, Van Reijsen FC *et al.* Antigen focussing by specific monomeric IgE bound to CD23 on EBV transformed B cells. Hum Immunol 1993; **37**:23–30.
5 Van der Heijden FL, Van Neerven RJJ, Van Katwijk M, Bos JD, Kapsenberg ML. Serum-IgE-facilitated allergen presentation in atopic disease. J Immunol 1993; **150**:3643–50.
6 Coffman RL, Carty J. A T cell activity that enhances polyclonal IgE production and its inhibition by interferon-gamma. J Immunol 1986; **136**:949.
7 Snapper CM, Paul WE. Interferon-gamma and B cell stimulatory factor-1 reciprocally regulate Ig isotype production. Science 1987; **236**:944–7.
8 Pène J, Rousset F, Brière F *et al.* IgE production by normal human B cells induced by alloreactive T cell clones is mediated by IL-4 and suppressed by IFN-gamma. J Immunol 1988; **141**:1218–24.
9 Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T-cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. J Immunol 1986; **136**:2348–57.
10 Wierenga EA, Snoek M, Bos JD, Jansen HM, Kapsenberg ML. Comparison of diversity and function of house dust mite-specific T lymphocyte clones from atopic and non-atopic donors. Eur J Immunol 1990; **20**:1519–26.
11 Mackie RM, Cobb S, Cochrane R, Thompson J. Total and specific IgE levels in patients with atopic dermatitis. Clin Exp Dernatol 1979; **4**:187–95.
12 Bruijnzeel-Koomen CAFM, Van Wichen DF, Spry CJF, Venge P, Bruijnzeel PLB. Active participation of eosinophils in patch test reactions to inhalent allergens in patients with atopic dermatitis. Br J Dermatol 1988; **118**:229–38.
13 Van Reijsen FC, Bruijnzeel-Koomen CAFM, Kalthoff FS *et al.* Skin-derived aeroallergen-specific T-cell clones of Th2 phenotype in patients with atopic dermatitis. J Allergy Clin Immunol 1992; **90**:184–93.
14 Sager N, Feldmann A, Schilling G, Kreitsch P, Neumann C. House dust mite-specific T cells in the skin of subjects with atopic dermatitis: frequency and lymphokine profile in the allergen patch test. J Allergy Clin Immunol 1992; **89**:801–10.
15 Ramb-Lindhauer C, Feldmann A, Rotte M, Neumann C. Characterization of grass pollen reactive T cell lines derived from lesional atopic skin. Arch Dermatol Res 1991; **283**:71–6.
16 Van der Heijden FL, Wierenga EA, Bos JD, Kapsenberg ML. High frequency of IL-4-producing $CD4^+$ allergen-specific T lymphocytes in atopic dermatitis lesional skin. J Invest Dermatol 1991; **97**:389–94.
17 Evavold BD, Allen PM. Separation of IL-4 production from Th cell proliferation by an altered T cell receptor ligand. Science 1991; **252**:1308–10.
18 Jenkins MK, Pardoll DM, Mizuguchi J, Chused TM, Schwartz RH. Molecular events in the induction of a nonresponsive state in interleukin 2-producing helper T-lymphocyte clones. Proc Natl Acad Sci USA 1987; **84**:5409–13.
19 Mueller DL, Chiodetti L, Bacon PA, Schwartz RH. Clonal anergy blocks the response to IL-4, as well as the production of IL-2, in dual-producing T helper cell clones. J Immunol 1991; **147**:4118–25.
20 Hanifin JM, Rajka G. Diagnostic features of atopic dermatitis. Acta Derm Venereol (Stockh) 1980; **Suppl. 92**:44–7.
21 Zubiaga AM, Munoz E, Huber BT. IL-4 and IL-2 selectively rescue Th cell subsets from glucocorticoid-induced apoptosis. J Immunol 1992; **149**:107–12.
22 Langhoff E, Steinman RM. Clonal expansion of human T lymphocytes initiated by dendritic cells. J Exp Med 1989; **169**:315–20.
23 Del Prete G, De Carli M, Mastromauro C *et al.* Purified protein derivative of *Mycobacterium tuberculosis* and excretory-secretory antigen(s) (TES) of *Toxocara canis* expand *in vitro* human T cells with stable and opposite (type 1 T helper or type 2 T helper) profile of cytokine production. J Clin Invest 1991; **88**:346–50.

24 Rolink AG, Melchers F, Palacios R. Monoclonal antibodies reactive with the mouse interleukin 5 receptor. J Exp Med 1989; **169**:1693–701.

25 Karasuyama H, Melchers F. Establishment of mouse cell lines which constitutively secrete large quantities of interleukin 2, 3, 4 or 5, using modified cDNA expression vectors. Eur J Immunol 1988; **18**:97–104.

26 Kurt Jones EA, Hamberg S, Ohara J, Paul WE, Abbas AK. Heterogeneity of helper/inducer T lymphocytes. I. Lymphokine production and lymphokine responsiveness. J Exp Med 1987; **166**:1774–87.

27 Fernandez-Botran R, Sanders VM, Mosmann TR, Vitetta ES. Lymphokine-mediated regulation of the proliferative response of clones of T helper 1 and T helper 2 cells. J Exp Med 1988; **168**:543–58.

28 Ebner C, Szepfalusi Z, Ferreira F *et al.* Identification of multiple T cell epitopes on Bet v I, the major birch pollen allergen, using specific T cell clones and overlapping peptides. J Immunol 1993; **150**:1047–54.

29 Lamb JR, Skidmore BJ, Green N, Chiller JM, Feldmann M. Induction of tolerance in influenza virus-immune T lymphocyte clones with synthetic peptides of influenza hemagglutinin. J Exp Med 1983; **157**:1434–47.

30 Jenkins MK, Schwartz RH. Antigen presentation by chemically modified splenocytes induces antigen-specific T cell unresponsiveness *in vitro* and *in vivo*. J Exp Med 1987; **165**:302–19.

31 Quill H, Schwartz RH. Stimulation of normal inducer T cell clones with antigen presented by purified Ia molecules in planar lipid membranes: specific induction of a long-lived state of proliferative nonresponsiveness. J Immunol 1987; **138**:3704–12.

32 Gajewski TF, Lancki DW, Stack R, Fitch FW. "Anergy" of Th0 helper T lymphocytes induces downregulation of Th1 characteristics and a transition to a Th2-like phenotype. J Exp Med 1994; **179**:481–91.

33 Sloan-Lancaster J, Evavold BD, Allen PM. Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. Nature 1993; **363**:156–9.

34 Burstein HJ, Shea CM, Abbas AK. Aqueous antigens induce *in vivo* tolerance selectively in IL-2 and IFN-gamma-producing (Th1) cells. J Immunol 1992; **148**:3687–91.

35 Burstein HJ, Abbas AK. *In vivo* role of interleukin 4 in T cell tolerance induced by aqueous protein antigen. J Exp Med 1993; **177**:457–63.

36 Reinhold U, Kukel S, Goeden B, Neumann U, Wehrmann W, Kreysel H. Interleukin 4 promotes the expansion of skin-infiltrating lymphocytes from atopic dermatitis *in vitro*. J Invest Dermatol 1991; **96**:370–5.

37 Symington FW, Brady W, Linsley PS. Expression and function of B7 on human epidermal Langerhans cells. J Immunol 1993; **150**:1286–95.

38 June CH, Ledbetter JA, Linsley PS, Thompson CB. Role of the CD28 receptor in T-cell activation. Immunol Today 1990; **11**:211–6.

39 Linsley PS, Brady W, Urnes M, Grosmaire LS, Damle NK, Ledbetter JA. CTLA-4 is a second receptor for the B cell activation antigen B7. J Exp Med 1991; **174**:561–9.

40 Linsley PS, Brady W, Grosmaire L, Aruffo A, Damle NK, Ledbetter JA. Binding of the B cell activation antigen B7 to CD28 costimulates T cell proliferation and interleukin 2 mRNA accumulation. J Exp Med 1991; **173**:721–30.

41 O'Hehir RE, Lamb JR. Human *in vitro* experimental model for $CD4^+$ T cell targeted immunotherapy to house dust mite. Ann Allergy 1993; **71**:317–21.

42 Strange P, Cooper KD, Hansen ER *et al.* T-lymphocyte clones initiated from lesional psoriatic skin release growth factors that induce keratinocyte proliferation. J Invest Dermatol 1993; **101**:695–700.