Clin. exp. Immunol. (1990) **81**, 417–422

Frequencies of interleukin-5 mRNA-producing cells in healthy individuals and in immunoglobulin-deficient patients, measured by *in situ* hybridization

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(Accepted for publication 16 March 1990)

SUMMARY

Interleukin-5 (IL-5) has previously been demonstrated to enhance immunoglobulin synthesis, especially IgA. Thus, it could be hypothesized that a defect production of IL-5 may cause immunoglobulin deficiency. We have analysed the frequency of IL-5 mRNA-producing cells in healthy adults and in patients with common variable immunodeficiency or selective IgA deficiency. Unstimulated lymphocytes were rarely found to synthesize IL-5 as measured by *in situ* hybridization. However, pokeweed mitogen and several other activating ligands induced the synthesis of IL-5 mRNA in peripheral blood and spleen lymphocyte cultures. After pokeweed mitogen activation, the number of IL-5 mRNA-producing cells most often peaked on day 3 with a maximal frequency of around 1–2% of mononuclear cells. In a kinetic study we were unable to detect any peak frequency differences between healthy controls (mean 0·44%) and 20 patients (mean 0·58%). Thus, although IL-5 has been reported to be an important regulator of IgA synthesis, a defect production does not seem to be the underlying mechanism in human immunoglobulin deficiency.

Keywords human interleukin-5 immunodeficiency IgA deficiency common variable immunodeficiency

INTRODUCTION

Interleukin-5 (IL-5) is a pleiotropic molecule with effects on eosinophil granulocytes (Sanderson, Campbell & Young, 1988) and on B (Takatsu *et al.*, 1988) as well as T lymphocytes (Takatsu *et al.*, 1987). Molecular cloning of mouse cDNAs for IL-5 was done by Kinashi *et al.* (1986), and was later followed by the isolation and characterization of human clones (Azuma *et al.*, 1986; Campbell *et al.*, 1987; Yokota *et al.*, 1987). The human gene for IL-5 has been mapped to chromosome 5q31 (Sutherland *et al.*, 1988). The effect of recombinant human IL-5 (rhIL-5) on immunoglobulin synthesis was first seen as a slight increase in IgM using human spleen cells (Azuma *et al.*, 1986). This observation was later confirmed using human peripheral blood lymphocytes (PBL) (Yokota *et al.*, 1987), where a strong effect on IgA secretion was also found (Yokota *et al.*, 1987; Pène *et al.*, 1988). However, a complete lack of stimulatory effects on human B cells has also been reported (Lopez *et al.*, 1988).

The effects of IL-5 on immunoglobulin production has been investigated in more detail in the mouse. An increased IgA synthesis in committed cells, in the absence of cell proliferation, has been reported (Harriman *et al.*, 1988; Beagley *et al.*, 1988). A pronounced IgA response in Peyer's patch cells was found after exposure to IL-5 (Harriman *et al.*, 1988; Beagley *et al.*, 1988). Recent experiments using 10 different cytokines have demonstrated that only IL-5 and interleukin-6 (IL-6) enhanced IgA synthesis in murine Peyer's patch cells (Beagley *et al.*, 1989). Furthermore, sterile IgH α-transcripts were induced by supernatants containing IL-5 (Stavnezer *et al.*, 1988). In view of the experimental evidence demonstrating that IL-5 is of importance for IgA synthesis, we have analysed the synthesis of IL-5 mRNA in patients with immunoglobulin deficiency. Since normal frequencies of IL-5-producing cells in humans had not been reported at the time of the study, cells from healthy blood donors were also investigated.

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MATERIALS AND METHODS

Lymphocyte preparation and culture conditions

PBL were prepared from heparinized venous blood or buffy coat (healthy blood donors) by flotation on Lymphoprep (Nyegaard, Oslo, Norway). Spleen cells were obtained from post-mortem kidney donors and thymocytes from patients undergoing thoracic surgery. Cells were cultured in RPMI 1640 medium (Flow Laboratories, Irvine, UK) supplemented with 10% heat-inactivated human serum in round-bottomed 5-ml

tubes (Falcon Plastics, Oxnard, CA) in a total volume of 1 ml. Different stimulatory reagents were added at the onset of culture. Pokeweed mitogen (PWM) and phytohaemagglutinin (PHA) were purchased from Sigma Chemical Co. (St Louis, MO) and concanavalin A (Con A) from Pharmacia (Uppsala, Sweden).

Cytocentrifuge preparations

Glass slides were immersed in 70% ethanol and air-dried. Cells suspended in balanced salt solution containing 10% fetal calf serum (FCS) were loaded on glass slides (10^5 cells/slide) with a cytocentrifuge. The cell smears were either frozen at $-20°C$ and stored, or fixed in paraformaldehyde (4% diluted in phosphate-buffered saline PBS) for 1 min, transferred into a solution of 70% ethanol, and subsequently stored at 4°C until used.

Preparation of the IL-5 probe

The *Pst*I–*Pst*I fragment of the cDNA clone ph.IL-5-30 (Azuma *et al.*, 1986) containing all the coding sequence for IL-5, was subcloned into a pGEM3 SP6/T7 vector (Promega Biotec, Madison, WI). The subclone was sequenced by the dideoxy method (Sanger, 1981) in order to verify that it had not been altered during subcloning. Linearized plasmids were used as templates for the synthesis *in vitro* of ^{35}S-labelled ssRNA probes complementary to the cellular IL-5 mRNA. RNA was also transcribed from the opposite direction (sense) and used as a negative control. An amount of 10^8–10^9 ct/min was incorporated into RNA per μg of RNA template. The size of the full-length probe mRNA was about 500 nucleotides.

In situ *hybridization*

In situ hybridization was carried out using a modification of the method described by Harper *et al.* (1986). Fixed slides prepared as described above were rinsed twice in 2 × SSC (= 0·3 M NaCl/0·03 M sodium citrate) and acetylated with 0·25% acetic anhydride in 0·1 M triethanolamine, pH 8, for 10 min. Subsequently, slides were rinsed in 2 × SSC and PBS and were thereafter immersed in 0·1 M Tris-HCl/0·1 M glycine, pH 7·0, for 30 min before hybridization. Twenty microliters of probe mixture, 10 μl of formamide containing 20% dextran sulphate, 2 μl of 20 × SSC containing 100 mM dithiothreitol, 2 μl of *Escherichia coli* tRNA, 2 μl of sheared herring sperm DNA (10 mg/ml), 2 μl of bovine serum albumin (BSA) (nuclease free; 20 mg/ml), and 1 μl of labelled RNA (10^6 ct/min per μl) were loaded on each slide, and hybridization was performed at 50°C for 3 h. The slides were washed three times with 50% formamide/2 × SSC at 52°C, rinsed in 2 × SSC, and treated with 30 μl of RNAse solution for 30 min at 37°C (100 μg of RNAse A (Sigma) plus 1 μg of RNAse T1 (Boehringer-Mannheim, FRG) per ml). The slides were rinsed twice in 2 × SSC and once in 50% formamide/2 × SSC at 52°C, rinsed again three times in 2 × SSC, dehydrated successively in 70%, 80%, and 90% ethanol, air-dried, and finally autoradiographed. Kodak nuclear track emulsion (NTB-2, Eastman Kodak, Rochester, NY) was melted at 43°C and diluted with an equal volume of water. The slides were dipped into the emulsion and allowed to solidify horizontally at room temperature for 3–4 h. The emulsion-coated slides were kept at 4°C for 7–14 days for exposure. The slides were developed in Kodak developer 19 at 15°C for 5 min. After a rinse in tap water, the fixation was carried out in Kodak fixer for 5 min, and the slides were washed with water for 30 min. The slides were counter-stained with Mayer's haematoxylin, washed twice with tap water, and air-dried. Stained and labelled cells were analysed by an Olympus BH-2 microscope (Tokyo, Japan) at a magnification of × 250. Cells with 10 or more silver grains over background were considered positive for IL-5 mRNA. Cells with different numbers of overlying grains were divided into two grain classes, < 100 grains and > 100 grains/cell, respectively. Autoradiographs were photographed using an Olympus C-35AD-4 camera.

Patients

Patients with IgA deficiency or with common variable immunodeficiency from our clinic were included in the study. They were selected on the basis of serum IgA levels. Most IgA-deficient patients had serum IgA < 0·02 g/*l* and all patients with common variable immunodeficiency had a serum IgA concentration of < 0·02 g/*l*.

RESULTS

Synthesis of IL-5 can be induced in human peripheral blood and spleen lymphocytes

In a first set of experiments cells from different organs were analysed for IL-5 secretion using *in situ* hybridization. Single-stranded ^{35}S-labelled RNA in both orientations was used as probes. Cells from human peripheral blood, spleen and thymus were studied using different stimulatory ligands. Both spleen and PBL could be activated to IL-5 mRNA synthesis by Con A,

Table 1. IL-5 mRNA-producing cells in peripheral blood and thymus (%)

Donor	Day	Organ	Activating ligand	Dose (μg/ml)	IL-5 positive cells*
1	2	PBL	—	—	0·01/ < 0·01
1	2	PBL	Con A	10	0·12/ < 0·003
1	2	PBL	PWM	20	0·08/ < 0·005
1	2	PBL	PHA	10	0·05/ < 0·005
1	3	PBL	Con A	10	0·06/ < 0·004
1	3	PBL	PWM	20	0·18/ < 0·002
2	2	PBL	—	—	< 0·005/ < 0·005
2	2	PBL	Con A	10	0·01/ < 0·005
2	2	PBL	PWM	20	0·06/ < 0·006
2	2	PBL	PHA	10	0·02/ < 0·008
2	3	PBL	Con A	10	0·09/ < 0·003
2	3	PBL	PWM	20	0·84/ < 0·002
2	3	PBL	PHA	10	0·29/ < 0·005
3	0†	PBL	—	—	< 0·01/ < 0·01
4	0	PBL	—	—	0·03/ < 0·03
5	2	Thymus	Con A	0·01	< 0·1/ < 0·1
5	2	Thymus	Con A	0·1	< 0·1/ < 0·1
5	2	Thymus	Con A	1	< 0·05/ < 0·05
5	2	Thymus	Con A	10	< 0·1/ < 0·1
6	2	Thymus	Con A	0·01	< 0·03/ < 0·005
6	2	Thymus	Con A	1	< 0·004/ < 0·003
6	2	Thymus	Con A	0·1	< 0·003/ < 0·005
6	2	Thymus	Con A	10	< 0·004/ < 0·006

* Per cent cells > 10 grains above background using an antisense IL-5 RNA probe/percent cells > 10 grains above background using a sense IL-5 RNA probe.

† Fresh unstimulated cells.

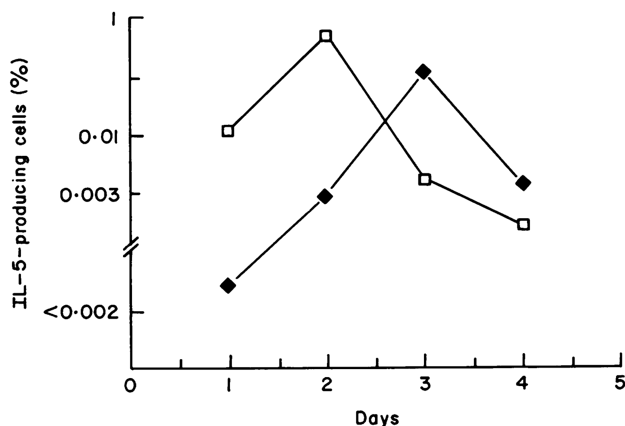

Fig. 1. Spleen cells from two cadaveric kidney donors were cultured with 20 μg of PWM/ml and assayed on days 1–4 for IL-5 mRNA-producing cells by the use of *in situ* hybridization.

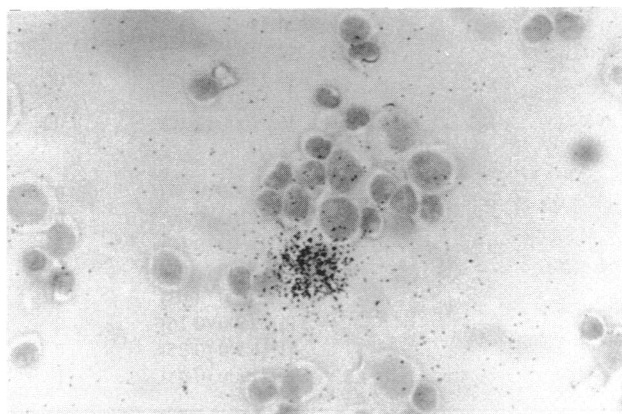

Fig. 2. Cell overlayed with silver grains after *in situ* hybridization using an IL-5 anti-sense ^{35}S-labelled ssRNA probe.

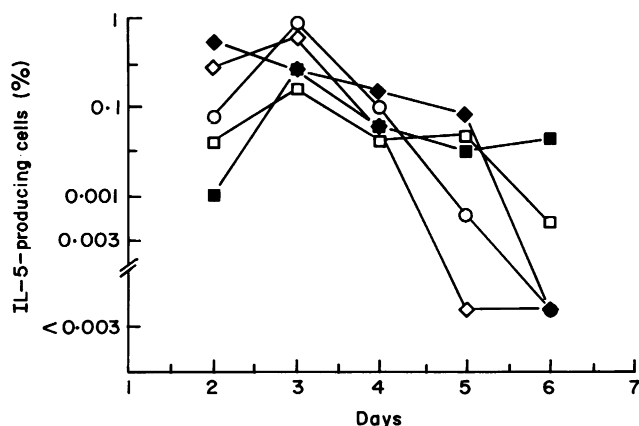

Fig. 3. PBL from four patients (□, ◆, ○ and ◇) with immunoglobulin deficiency and one control (■) were cultured with 20 μg of PWM/ml and assayed on days 2–6 for IL-5 mRNA-producing cells by the use of *in situ* hybridization.

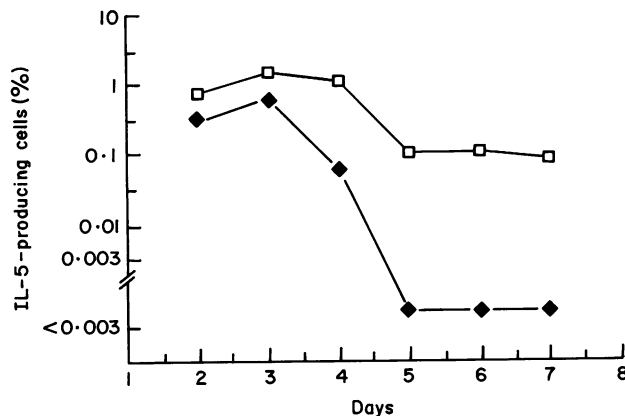

Fig. 4. Cells obtained and cultured on two different occasions (□, experiment 1; ■, experiment 2) from one patient with common variable immunodeficiency were cultured with 20 μg of PWM/ml and assayed on days 2–7 for IL-5 mRNA producing cells by *in situ* hybridization.

PHA and PWM, whereas we were unable to detect any synthesis in cells from two human thymuses activated with Con A and only rarely in unstimulated cells (Table 1, Fig. 1, and data not shown). Figure 2 is an autoradiograph with a typical blast-shaped cell with overlaying silver grains. Cells with > 10 grains above background were only observed using the anti-sense RNA probe (Table 1).

Based on these initial findings, PWM was chosen as a stimulatory agent. In order to study conditions for optimal activation, different cell densities and various concentrations of PWM were analysed. The results indicated that the concentrations that we normally apply for optimal immunoglobulin synthesis (10^6 cells/ml; 20 μg of PWM/ml) also resulted in maximal IL-5 production (Table 2, and data not shown). The peak response for IL-5 was obtained on days 2–4 (Tables 1 and 2, Figs 1 and 3).

Frequency of IL-5-secreting cells in immunodeficiency

Since the peak response day varied, cells from healthy adults and from immunoglobulin-deficient patients were stimulated with PWM and assayed on days 2–6. Figure 3 depicts the typical kinetics of the PWM-induced IL-5 mRNA synthesis. Table 2 summarizes the analysis and demonstrates that there were no obvious differences between healthy adult blood donors and the 20 patients with low or undetectable IgA levels. The mean frequency of IL-5-producing cells on the peak day was 0·53 for 13 IgA-deficient patients; 0·9% for five patients with common variable immunodeficiency; and 0·44% for eight healthy controls. In order to study whether there was a difference in the IL-5 mRNA content/cell, cells were subdivided according to the number of grains into two groups, one with < 100 grains/cell and one with > 100 grains/cell. In most cases cells with < 100 grains dominated, although in two out of 13 IgA donors with selective IgA deficiency and in one healthy blood donor there was an inverted ratio with a higher frequency of cells overlayed with > 100 silver grains (Table 2). Furthermore, the mean ratios in patients and controls (> 100 grains per cell/ < 100 grains per cell) were similar: selective IgA deficiency 0·51; common variable immunodeficiency 0·40; and controls 0·36. The maximal frequencies of IL-5 mRNA-containing cells were around 2% of cultured cells. Table 3 also demonstrates that one patient

Table 2. Frequency of IL-5-producing cells in immunodeficiency patients

Immunodeficiency*	Patient	Age (years)	Sex	Serum immunoglobulin levels (g/*l*)†			Salivary IgA‡	Day of IL-5 peak§	Total frequency of IL-5 cells¶	Cells with >100/cells <100 grains
				IgM	IgG	IgA				
IgA deficiency										
	ÅG	20	F	2·2	22	0·03	<	3	7/16800 (0·042)	0·17
	AA	23	F	1·2	23	<0·02	<	3	31/33000 (0·94)	0·35
	RG	28	M	1·4	16	<0·02	Traces	3	9/46000 (0·020)	0·29
	LJ	29	F	1·9	30	<0·02	<	3	11/33100 (0·033)	0·38
	PO	31	M	0·4	8·1	<0·02	<	3	84/9600 (0·87)	0·33
	SB	36	M	0·9	13	<0·02	<	3	45/7600 (0·59)	0·31
	KE	39	F	1·5	19	0·03	<	3	150/24500 (0·57)	0·11
	AH	40	F	1·4	22	<0·02	<	4	1/19300 (0·005)	<0·5
	BE	40	F	1·9	18	<0·02	<	3	21/25500 (0·08)	2·0
	LL	45	F	1·7	17	<0·02	<	2	72/5280 (0·14)	0·26
	SG	55	M	1·6	19	<0·02	<	4	23/28800 (0·08)	0·35
	MV	57	F	1·9	18	0·03	<	2	40/12800 (0·31)	0·74
	BN	62	F	0·8	19	<0·02	<	3	61/2190 (0·28)	1·3
Low IgA	GW	44	F	2·2	16	0·1	+	3	13/8160 (0·16)	0·30
CVI	MK	34	M	0·1	1·2	<0·02	Traces	3	422/28800 (0·15)	0·30
	RB	51	M	0·3	2·2	<0·02	<	3	59/6700 (0·88)	0·34
	KS	55	F	0·3	0·5	<0·02	<	3	40/23000 (0·17)	0·25
	IG	66	F	0·3	2·8	<0·02	<	3	33/13100 (0·25)	0·83
	ES	73	F	0·9	0·5	<0·02	<	4	436/25900 (0·17)	0·28
IgG1 + low IgG3 and IgA	MA	64	F	1·5	4·2	0·3	+	2	36/31000 (0·12)	0·8
Control	1	>20	—	—	—	—	—	3	61/19200 (0·37)	0·20
Control	2	>20	—	—	—	—	—	3	208/31700 (0·66)	0·24
Control	3	>20	—	—	—	—	—	2	22/21600 (0·10)	0·47
Control	4	>20	—	—	—	—	—	3	12/13400 (0·084)	0·33
Control	5	>20	—	—	—	—	—	4	1/29300 (0·03)	>1
Control	6	>20	—	—	—	—	—	3	316/19500 (0·16)	0·10
Control	7	>20	—	—	—	—	—	3	41/16200 (0·25)	0·37
Control	8	>20	—	—	—	—	—	3	99/21900 (0·45)	0·19

* Selective IgA deficiency; and common variable immunodeficiency (CVI).

† IgG subclass levels were normal in all patients with the exception of the five patients with CVI, patient MA with IgG1 of 2·7 (normal >4·2) and IgG3 of 0·3 (normal >0·3) g/*l*, and patient MV with IgG3 of 0·2 g/*l*. Normal values are for IgM 0·3–2·5, IgG 7–16 and IgA 0·5–3·3 g/*l*.

‡ < denotes undetectable IgA.

§ PWM stimulated cultures from days 2–6 were analysed.

¶ Total number of cells counted, given as cells with > 10 overlaying grains above background/negative cells. In parenthesis, per cent positive cells.

with selective IgA deficiency and one healthy donor had very low levels (<0·01%) of IL-5-producing cells. In order to study the extent to which experimental variation could contribute, cells from a few donors were stimulated with PWM on different occasions. Figure 4 depicts one such experiment using cells from a patient with common variable immunodeficiency (patient MK). The peak response occurred on the same day and had a similar magnitude. However, there was a pronounced difference in the post-peak response. Thus, an experimental variation in the late response was observed.

DISCUSSION

Our findings demonstrate that human peripheral blood and spleen lymphocytes can be stimulated to IL-5 synthesis, and, furthermore, that a global defect synthesis of mRNA for IL-5 is not a common cause for IgA deficiency. This was observed in a kinetic study of 13 patients with selective IgA deficiency as well as in five donors with common variable immunodeficiency having undetectable IgA and, furthermore, in one individual with a low but detectable IgA level and finally in one patient with a relative IgG1 deficiency in combination with a serum IgA and IgG3 at the lower normal level. Cells from 15 additional patients were analysed on days 2 or 3 only after PWM stimulation, with similar results (unpublished).

Synthesis of IL-5 was rarely observed in fresh or cultured unstimulated lymphocytes. In the mouse it has been shown that the helper T cells responsible for IL-4 and IL-5 production are present as precursors that require priming before they develop into lymphokine-secreting cells (Swain *et al.*, 1988). We found a similar maximum frequency of IL-5-synthesizing cells as previously reported for IL-4 in humans (Lewis *et al.*, 1988), although the IL-5 mean values were somewhat lower. Lewis *et al.* (1988) found that 1–2% of human peripheral blood T cells

could be induced to IL-4 synthesis. Since the majority of cells stimulated in cultures with PWM are T cells (Greaves, Janossy & Doenhoff, 1974; Hammarström *et al.*, 1979), the number of IL-4 producers thus seems to be in the same order as the maximal number of cells synthesizing IL-5. We do not know whether the same cells make both IL-4 and IL-5. In the mouse the Th2 subset has been demonstrated to produce both IL-4 and IL-5 in addition to other interleukins (Mosmann *et al.*, 1986; Mosmann & Coffman, 1989). However, in humans, T cells do not seem to be divisible into the Th1 and Th2 subsets, but rather to be able to express several different combinations of lymphokines (Umetsu *et al.*, 1988; Paliard *et al.*, 1988).

It has been reported that primarily CD4 cells produce IL-4 (Paliard *et al.*, 1988). Although it seems likely that removal of CD8 cells prior to culture might have resulted in a higher frequency of IL-5-producing cells, we chose not to apply this method. Thus, it could be argued that the defect in immunodeficiency is caused by an increased inhibitory effect of the CD8 positive T cell population on IL-5 synthesis rather than by an absence of cells capable of IL-5 synthesis.

A similar maximum frequency of cells producing IL-5 was found in spleen and peripheral blood. The fact that we could not detect any synthesis in the limited number of thymocytes analysed, does not exclude the existence of IL-5-producing cells in the thymus. Thus, only two thymuses were studied, and it may well be that activating ligands other than Con A could be more effective, although there were no obvious differences between Con A and PWM as IL-5 inducers for PBL. However, it has previously been observed for IL-4 in mice that only fetal but not adult thymocytes make IL-4 (Sideras *et al.*, 1988).

Although there was no difference in IL-5 mRNA synthesis between healthy controls and immunodeficient patients there was an inter-individual variation. This did not seem to be associated with a poor PWM response as measured by ^{3}H-thymidine incorporation or number of blasts in the cultures (unpublished data). Since IL-5 has been reported to be a very potent inducer of eosinophil differentiation (Sanderson *et al.*, 1988), the eosinophil counts were compared with the PWM-induced IL-5 synthesis. However, a preliminary analysis indicated that there was no obvious correlation between eosinophil numbers and the frequency of IL-5 synthesis after PWM activation (unpublished data).

As demonstrated in Fig. 4, a pronounced decrease in the IL-5 mRNA synthesis was found in the late phase of the PWM-induced IL-5 response in certain experiments. The dominating pattern was a gradual increase prior to the peak, followed by a sharp decrease after the peak (Fig. 3). The most extreme decrease (> 500-fold) was seen in a donor with 1·7% of positive cells on day 3 but with < 0·003% of cells reacting with the IL-5 anti-sense probe on day 4. We are not aware of any differences in the experimental conditions in experiments with high *versus* low IL-5 late responses. Thus, there were no differences in ^{3}H-thymidine incorporation or culture cell numbers indicative of poor culture conditions in experiments with low IL-5 late phase responses (unpublished data). Although this rapid decrease in the cultures seems to be caused by *in vitro* conditions, the underlying mechanism could be of interest. It is known that AT-rich stretches in the 3′ untranslated region of several interleukin mRNA species confers a short half-life (Cosman, 1988). Since such AT-rich stretches seem to be present in IL-5 mRNA (Azuma *et al.*, 1986), conditions resulting in an inhibition of IL-5 transcription would thus be expected to rapidly reduce the IL-5 mRNA cell content. The early phase of the response (day 1) has not been investigated in detail. However, in view of the fact that we rarely found IL-5 mRNA-producing cells in fresh lymphocyte samples (Table 1, and unpublished data), the early increase may be rapid.

The mechanisms underlying human immunoglobulin deficiency in most cases still remain elusive (Smith & Hammarström, 1987). We and others have described individuals with homozygous deletions of immunoglobulin constant region genes, but these are extremely rare conditions (Lefranc, Lefranc & Rabbitts, 1982; Hammarström, de Lange & Smith, 1987; Smith *et al.*, 1989). Furthermore, in children of patients with IgA deficiency, the silent $C\alpha$-genes are re-expressed indicating that not only the coding sequences are intact but also that the IgCH stretches involved in the regulation of expression seem to be accurate (Hammarström *et al.*, 1987). Molecules regulating the expression of immunoglobulins would thus be expected to be involved in the pathogenesis. Interleukins are obvious candidates for such molecules. However, it has recently been shown that the addition of IL-2 and IL-4 only had minor effects on immunoglobulin synthesis in patients with common variable immunodeficiency (Farrant *et al.*, 1989). However, these and our findings on IL-5 do not completely rule out these interleukins as factors involved in the disease. Thus, although the addition of interleukins did not result in increased immunoglobulin synthesis, it is possible that it is a precursor population not present in the lymphocyte cultures that is dependent on a particular interleukin for differentiation. In the case of *in situ* hybridizations, this objection may not be relevant; instead, we do not know whether the mRNA is properly translated. Furthermore, neither of these approaches excludes defects in the receptors for interleukins in the relevant target population. The murine receptor for IL-5 has recently been characterized (Mita *et al.*, 1988, 1989) and the isolation of the human counterpart will enable such studies in immunodeficiency patients.

ACKNOWLEDGMENTS

This work was supported by the Swedish Medical Research Council, the Sigurd and Elsa Golje Memorial Foundation, the Ellen, Walter and Lennart Hesselman Foundation and the WHO Directors Initiative's fund. The skilful technical assistance of Ms Silvia Nava and Ms Sirkka Hellman is gratefully acknowledged. We are indebted to Prof. T. Honjo for providing the ph.IL-5-30 cDNA clone.

REFERENCES

Azuma, T., Tanabe, T., Konishi, M., Noma, T., Matsuda, F., Yaoita, Y., Takatsu, K., Hammarström, L., Smith, C.I.E., Severinson, E. & Honjo, T. (1986) Cloning of a cDNA for human T cell replacing factor (interleukin 5) and comparisons with the murine homologue. *Nucl. Acids Res.* **14**, 9149.

Beagley, K.W., Eldridge, J.H., Kiyono, H., Everson, M.P., Koopman, W.J., Honjo, T. & McGhee, J.R. (1988) Recombinant murine IL-5 induces high rate IgA synthesis in cycling IgA-positive Peyer's patch B cells. *J. Immunol.* **141**, 2035.

Beagley, K.W., Eldridge, J.H., Lee, J., Kiyono, H., Everson, M.P., Koopman, W.J., Hirano, T., Kishimoto, T. & McGhee, J.R. (1989) Interleukins and IgA synthesis. Human and murine interleukin 6 induce high rate IgA secretion in IgA-committed B cells. *J. exp. Med.* **169**, 2133.

Campbell, H.D., Tucker, W.Q.J., Hort, Y., Martinson, M.E., Mayo E.J., Clutterbuck, G., Sanderson, C.J. & Young, I.G. (1987)

Molecular cloning and expression of the gene encoding human eosinophil differentiation factor (interleukin-5). *Proc. natl Acad. Sci. USA*, **84**, 6629.

COSMAN, D. (1987) Control of messenger RNA stability. *Immunol. Today*, **8**, 16.

FARRANT, J., BRYANT, A., ALMANDOZ, F., SPICKETT, G., EVANS, S.W. & WEBSTER, A.D.B. (1989) B cell function in acquired "common-variable" hypogammaglobulinemia: Proliferative responses to lymphokines. *Clin. Immunol. Immunopathol.* **51**, 196.

GREAVES, M.F., JANOSSY, G. & DOENHOFF, M.J. (1974) Selective triggering of human T and B lymphocytes in vitro by polyclonal mitogens. *J. exp. Med.* **140**, 1.

HAMMARSTRÖM, L., DE LANGE, G. & SMITH, C.I.E. (1987) IgA2 allotypes in IgA deficiency. Re-expression of the silent IgA2m2 allotype in the children of IgA deficient patients. *J. Immunogenet.* **14**, 197.

HAMMARSTRÖM, L., BIRD, A.G., BRITTON, S. & SMITH, C.I.E. (1979) Pokeweed mitogen induced differentiation of human B cells: evaluation by a protein A haemolytic plaque assay. *Immunology*, **38**, 131.

HARPER, M., MARSELLE, L., WONG-STAAL, F. & GALLO, R.C. (1986) Detection of lymphocytes expressing human T-lymphotropic virus type III in lymph nodes and peripheral blood from infected individuals by in situ hybridization. *Proc. natl Acad. Sci. USA*, **83**, 772.

HARRIMAN, G.R., KUNIMOTO, D.Y., ELLIOTT, J.F., PAETKAU, V. & STROBER, W. (1988) The role of IL-5 in IgA B cell differentiation. *J. Immunol.* **140**, 3033.

KINASHI, T., HARADA, N., SEVERINSON, E., TANABE, T., SIDERAS, P., AZUMA, C., TOMINAGA, A., BERGSTEDT-LINDQVIST, S., TAKASHI, M., KONISHI, M., MATSUDA, F., YAOITA, Y., TAKATSU, K. & HONJO, T. (1986) Cloning of complementary DNA encoding T-cell replacing factor and identity with B-cell growth factor II. *Nature*, **324**, 70.

LEFRANC, M.P., LEFRANC, G. & RABBITTS, T.H. (1982) Inherited deletion of immunoglobulin heavy constant region genes in normal human individuals. *Nature*, **300**, 760.

LEWIS, D.B., PRICKETT, K.S., LARSEN, A., GRABSTEIN, K., WEAVER, M. & WILSON, C.B. (1988) Restricted production of interleukin 4 by activated human T cells. *Proc. natl Acad. Sci. USA*, **85**, 9743.

LOPEZ, A.F., SANDERSON, C.J., GAMBLE, J.R., CAMPBELL, H.D., YOUNG, I.G. & VADAS M.A. (1988) Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J. exp. Med.* **167**, 219.

MITA, S., HARADA, N., NAOMI, S., HITOSHI, Y., SAKAMOTO, K., AKAGI, M., TOMINAGA, A. & TAKATSU, K. (1988) Receptors for T cell-replacing factor/interleukin 5. Specificity, quantitation, and its implication. *J. exp. Med.* **168**, 863.

MITA, S., TOMINAGA, A., HITOSHI, Y., SAKAMOTO, K., HONJO, T., MASANOBU, A., KIKUCHI, Y., YAMAGUCHI, N. & TAKATSU, K. (1989) Characterization of high-affinity receptors for interleukin 5 on interleukin 5-dependent cell lines. *Proc. natl Acad. Sci. USA*, **86**, 2311.

MOSMANN, T.R. & COFFMAN, R.L. (1989) Th1 and Th2 cells: different patterns of lymphokine secretion had two different functional properties. *Annu. Rev. Immunol.* **7**, 145.

MOSMANN, T.R., CHERWINSKI, H., BOND, M.W., GIEDLIN, M.A. & COFFMAN, R.L. (1986) Two types of murine helper T cell clones. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136**, 2348.

PALIARD, W., DE WAAL MALEFIJT, R., YSSEL, H., BLANCHARD, D., CHRÉTIEN, I., ABRAMS, J., DE VRIES, J. & SPITS, H. (1988) Simultaneous production of IL-2, IL-4, and IFN-γ by activated human CD4+ and CD8+ T cell clones. *J. Immunol.* **141**, 849.

PÈNE, J., ROUSSET, F., BRIÈRE, F., CHRÉTIEN, I., WIDEMAN, J., BONNEFOY, J.Y. & DE VRIES, J.E. (1988) Interleukin 5 enhances interleukin 4-induced IgE production by normal human B cells. The role of soluble CD23 antigen. *Eur. J. Immunol.* **18**, 929.

SANDERSON, C.J., CAMPBELL, H.D. & YOUNG, I.G. (1988) Molecular and cellular biology of eosinophil differentiation factor (interleukin-5) and its effects on human and mouse B cells. *Immunol. Rev.* **102**, 29.

SANGER, F. (1981) Determination of nucleotide sequences in DNA. *Science*, **214**, 1205.

SIDERAS, P., FUNA, K., ZALCBERG-QUINTANA, I., XANTHOPOULOS, K.G., KISIELOW, P. & PALACIOS, R. (1988) Analysis by in situ hybridization of cells expressing mRNA for interleukin 4 in the developing thymus and in peripheral lymphocytes from mice. *Proc. natl Acad. Sci. USA*, **85**, 218.

SMITH, C.I.E. & HAMMARSTRÖM, L. (1987) Cellular basis of immunodeficiency. *Ann. clin. Res.* **19**, 220.

SMITH, C.I.E., HAMMARSTRÖM, L., HENTER, J.-I. & DE LANGE, G. (1989) Molecular and serological analysis of IgG1 deficiency caused by new forms of the constant region of the IgH chain gene deletions. *J. Immunol.* **142**, 4514.

STAVNEZER, J., RADCLIFFE, G., LIN, Y-C., NIETUPSKI, J., BERGGREN, L., SITIA, R. & SEVERINSON, E. (1988) Immunoglobulin heavy-chain switching may be directed by prior induction of transcripts from constant-region genes. *Proc. natl Acad. Sci. USA*, **85**, 7704.

SUTHERLAND, G.R., BAKER, E., CALLEN, D.F., CAMPBELL, H.D., YOUNG, I.G., SANDERSON, C.J., GARSON, O.M., LOPEZ, A.F. & VADAS, M.A. (1988) Interleukin-5 is at 5q31 and is deleted in the 5q- syndrome. *Blood*, **71**, 1150.

SWAIN, S.L., MCKENZIE, D.T., WEINBERG, A.D. & HANCOCK, W. (1988) Characterization of T helper 1 and 2 cell subsets in normal mice: Helper T cells responsible for IL-4 and IL-5 production are present as precursors that require priming before they develop into lymphokine-secreting cells. *J. Immunol.* **141**, 3445.

TAKATSU, K., KIKUCHI, Y., TAKAHASHI, T., HONJO, T., MATSUMOTO, M., HARADA, N., YAMAGUCHI, N. & TOMINAGA, A. (1987) Interleukin 5, a T-cell-derived B-cell differentiation factor also induces cytotoxic T lymphocytes. *Proc. natl Acad. Sci. USA*, **84**, 4234.

TAKATSU, K., TOMINAGA, A., HARADA, N., MITA, S., MATSUMOTO, M., TAKAHASHI, T., KIKUCHI, Y. & YAMAGUCHI, N. (1988) T cell-replacing factor (TRF)/interleukin 5 (IL-5): molecular and functional properties. *Immunol. Rev.* **102**, 107.

UMETSU, D.T., JABARA, H.H., DEKRUYFF, R.H., ABBAS, A.K., ABRAMS, J.S. & GEHA, R.S. (1988) Functional heterogeneity among human inducer T cell clones. *J. Immunol.* **140**, 4211.

YOKOTA, T., COFFMAN, R.L, HAGIWARA, H., RENNICK, D., TAKEBE, Y., YOKOTA, K., GEMMELL, L., SHRADER, B., YANG, G., MEYERSON, P., LUH, J., HOY, P., PENE, J., BRIERE, F., BANCHEREAU, J., DE VRIES, J., LEE, F., ARAI, N. & ARAI, K. (1987) Isolation and characterization of lymphokine cDNA clones encoding mouse and human IgA-enhancing factor and eosinophil colony stimulating factor activities: relationship to interleukin 5. *Proc. natl Acad. Sci. USA*, **84**, 7388.