

## Analysis of intrathyroidal cytokine production in thyroid autoimmune disease: thyroid follicular cells produce interleukin-1 $\alpha$ and interleukin-6

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### SUMMARY

Cytokine production was studied in thyroid tissue from patients with Graves' disease, Hashimoto's thyroiditis and non-toxic goitre. The expression of interferon  $\gamma$ , tumour necrosis factor  $\alpha$  and  $\beta$ , interleukin-1 $\alpha$  and  $\beta$ , interleukin-6 and platelet-derived growth factor A chain was assessed by slot-blot analysis of the respective mRNA in freshly isolated tissue samples. All seven cytokines were detected in patients of all groups. Although the respective mRNA levels were, in general, higher in thyroid autoimmune disorders, this appeared to relate to the degree of the lymphocytic infiltration of the thyroid gland at the time of surgery. Purified thyroid follicular cells expressed high levels of interleukin-1 $\alpha$  and interleukin-6 mRNA and when established in primary culture, purified thyroid follicular cells from Graves' disease as well as non-toxic goitre produced interleukin-1 $\alpha$  and interleukin-6 bioactivity spontaneously. In the case of interleukin-1 this could be further augmented by addition of lipopolysaccharide to the thyroid follicular cell cultures. These results demonstrate that the lymphocytic infiltrate found in autoimmune and non-autoimmune thyroid disorders is associated with cytokine production. Additionally we have shown that intrathyroidal cytokine production is not restricted to thyroid-infiltrating mononuclear cells, but may also involve thyroid follicular cells both *in vivo* and *in vitro*. The cytokines produced by thyroid follicular cells may have an important role in stimulating autoantigen specific T cells *in vivo* as both interleukin-1 and interleukin-6 facilitate T cell activation.

**Keywords** thyroid autoimmunity cytokines

### INTRODUCTION

Cytokines are known to mediate processes that are likely to be important in both the initiation and perpetuation of thyroid autoimmunity, including the induction of HLA class II expression on thyroid epithelial cells by interferon-gamma (IFN- $\gamma$ ) (Todd *et al.*, 1985). This enables thyroid epithelial cells to present peptide and autoantigens to the appropriate T cells (Londei, Botazzo & Feldmann, 1984; Londei *et al.*, 1985; Grubeck-Loebenstein *et al.*, 1988), affecting target cell function and viability [interleukin-1 (IL-1)] (Bendtsen *et al.*, 1986; Krogh-

Rasmussen *et al.*, 1987), stimulating T cell growth [IL-1 and interleukin-6 (IL-6)] (Uyttenhove, Coulie & Van Snick, 1988; Houssiau *et al.*, 1988), and inducing the production of autoantibodies (IL-6) (Hirano *et al.*, 1986). However, little is known about cytokine production at the sites of autoimmune processes. Recent work from our laboratory has demonstrated, at the mRNA level, the increased production of IL-1 $\alpha$  and  $\beta$ , tumour necrosis factor (TNF)- $\alpha$  and  $\beta$  (TNF- $\beta$  is also known as lymphotoxin), interleukin-2 (IL-2) and IFN- $\gamma$  by cells isolated from rheumatoid joints (Buchan *et al.*, 1988a, b). However, in the absence of appropriate control tissues it is not yet clear whether the phenomena described were specifically linked to autoimmune disorders or have to be regarded as correlates of lymphocytic infiltration, activation and tissue inflammation.

Non-autoimmune thyroid disorders such as non-toxic goitre or thyroid carcinoma are frequently accompanied by a lymphocytic infiltration of the thyroid gland (Lloyd *et al.*, 1985; Grubeck-Loebenstein *et al.*, 1986; Lucas-Martin *et al.*, 1988), which in contrast to Graves' disease (GD) infiltrates, lacks

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autoantigen specificity (Grubeck-Loebenstein *et al.*, 1988). The thyroid seemed therefore a good system in which to compare cytokine production in autoimmune and non-autoimmune tissues. We have used slot-blot analysis of mRNA from surgical specimens from GD, Hashimoto's thyroiditis (HT), and non-toxic goitre and have looked at the capacity of purified thyroid epithelial cells to make cytokines *in vitro*.

## MATERIALS AND METHODS

### Patients

Thyroid tissue was obtained at surgery from nine women (mean age  $38 \pm 17$  years) with GD, two women (aged 50 and 42 years) with HT, and seven patients (five women, two men. Mean age  $50 \pm 16$  years) with multinodular non-toxic goitre. Patients with GD, underwent operation because of recurrent hyperthyroidism in spite of continuous drug treatment. Onset of the disease had been maximally 2 years prior to surgery in all cases. All GD patients were euthyroid under low-dose therapy with methimazole or propylthiouracil. Patients with HT underwent operation because of local symptoms in one and for suspected lymphoma in the other case. Both were euthyroid under thyroid hormone therapy. Patients with non-toxic goitre underwent operation for long-standing goitre with local symptoms. They were all euthyroid and had been taken off thyroid hormone suppression therapy at least 6 weeks prior to surgery. The amount of lymphocytic infiltration was assessed in routine histology slides and was classified semiquantitatively. In three out of nine GD and in the two HT patients extensive lymphocytic infiltrations was seen. In three of nine GD and in three of seven non-toxic goitre patients moderate lymphocytic aggregates could be seen, whereas in three out of nine GD and in three out of seven non-toxic goitre patients little or no infiltrate could be detected.

### Preparation of thyroid tissue

Tissue samples were dispersed with collagenase (Cooper Biomedical Collagenase, type IV, 5 mg/ml, in RPMI 1640 medium containing 15% fetal calf serum, FCS, both from GIBCO, Paisley, UK), for 3 h and pipetted through a 200- $\mu$ m mesh. The red blood cells were lysed with an ammonium chloride buffer. The resulting cell suspensions were used directly for mRNA analysis. Thyroid follicular cells (TFC) were purified as previously described (Grubeck-Loebenstein *et al.*, 1988). Briefly, TFC were purified on the basis of their strong adherence to plastic. Cells were incubated in RPMI supplemented with 10% FCS at 37°C in an atmosphere of 5% CO<sub>2</sub> and 95% humidity. After 16 h, non-adherent and loosely adherent cells were removed by vigorous washing and the remaining adherent cells were removed from their plastic support with trypsin (GIBCO trypsin solution, 0.25%). This cell population consisted of >95% TFC, as estimated by staining with a serum from a patient with GD, containing a high titre of microsomal antibodies, as previously described (Londei, Bottazzo & Feldmann, 1985). For additional depletion of contaminating cells TFC were incubated with a mixture of monoclonal antibodies against macrophages (LEU M1, Becton-Dickinson & Co, Oxnard, CA) and endothelial cells (EN 4, donated by M. Tai, St. Georges Hospital, London, UK) for 45 min on ice, washed and re-incubated with baby rabbit complement for 60 min at 37°C. After that, TFC purity was 98%. Fibroblast contamination was

assessed by staining with anti-human Thy 1 monoclonal antibody F15-42-1 (donated by J. Fabre, Blond McIndoe Centre for Medical Research, East Grinstead, UK) and was <2% in all cases. Thyroid-infiltrating mononuclear cells were purified from the non-adherent cell population over a Ficoll-Hypaque gradient (Lymphoprep, Nycomed, Torshov, Norway).

### Cell lines

A human umbilical vein endothelial cell line and a fibroblast cell line derived from human umbilical vein were kindly provided by Dr Gareth Howells (Charing Cross Sunley Research Centre).

### Slot-blot RNA analysis

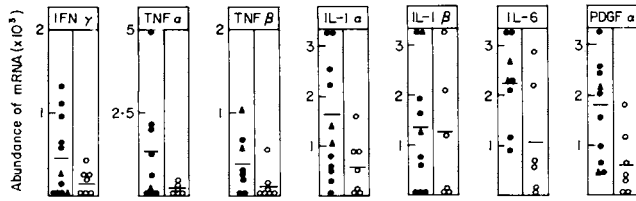
Total cytoplasmic RNA was extracted from cells as described previously (Buchan *et al.*, 1988). RNA was denatured and blotted onto nitrocellulose filters. The filters were then probed under stringent conditions with cDNA probes for IFN- $\gamma$ , IL-2, TNF- $\alpha$ , TNF- $\beta$ , IL-1 $\alpha$  and  $\beta$  (Buchan *et al.*, 1988a, b and IL-6 (Hirano *et al.*, 1988). The platelet-derived growth factor (PDGF) A probe was a 1.4 kbp *EcoRI* insert (Betsholtz *et al.*, 1986), kindly donated by Dr C. Betsholtz. The control probe 7B6, a 708-kbp *PstI-DraI* fragment from p7B6 detects a species of mRNA, which does not fluctuate during the cell cycle or in response to cell activation (Kaczmarek, Calabretta & Baserga, 1985; Buchan *et al.*, 1988a). The blots were stripped of the previous probe by washing in 5 mM Tris pH 8, 0.2 mM EDTA, 0.05% sodium pyrophosphate and 0.1 Denhardt's solution at 65°C for 1–2 h; stripping efficiency was monitored by autoradiography. The blots were then re-hybridized with the next probe.

### Quantitative analysis of slot blots

In order to avoid interassay variations, all samples to be finally compared were hybridized in one experiment. The autoradiographs were scanned on a densitometer (Joyce Loebl, Chromoscan III) and the peak integral values from the linear range of the scan (arbitrary scanning units) were used to define the amount of lymphokine mRNA per sample. The values were adjusted for the amount of mRNA per sample by normalization to 7B6 (Buchan *et al.*, 1988a).

### Bioassays

IL-1 levels were determined using the thymocyte co-mitogenic assay as described previously (Buchan *et al.*, 1988b) and IL-6 levels were measured using the B9 hybridoma growth assay (Aarden *et al.*, 1987). Values for IL-1 levels were expressed as either ct/min or as a percentage of phytohaemagglutinin (PHA) induced <sup>3</sup>H-thymidine uptake after addition of conditioned supernatants from lipopolysaccharide (LPS) untreated thyroid follicular cells (TFC), which was considered as 100%; ct/min were normalized to the slightly varying TFC numbers in the different samples, cell numbers referring to the end of the culture period. In some experiments supernatants were additionally assayed after a 60 min pre-incubation at room temperature in the presence of neutralizing, rabbit polyclonal antibodies specifically directed against IL-1 $\alpha$  and IL-1 $\beta$  (a generous gift from Steven Gillis and Christopher Henney, Immunex, Seattle) or goat anti IL-6 (kindly provided by L. A. Aarden, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, Amsterdam). Levels of IL-6 are expressed as either ct/



**Fig. 1.** Slot-blot analysis of cytokine mRNA in unpurified thyroid cells from patients with thyroid autoimmune disease (●, Graves' disease; ▲, Hashimoto's thyroiditis) and non-toxic goitre (○). Numerical values were determined as described in Materials and Methods. Not all patients samples could be quantified for the presence of some cytokine mRNAs and these have been excluded from the analysis. All numerical values are adjusted to the total amount of RNA per sample, assessed by reprobing of the filters for the cell cycle independent mRNA, 7B6.

min or units/ml, determined by comparison with a standard curve (dilutions of a known amount of recombinant IL-6, 1 unit of IL-6 is defined as that amount of IL-6 that induces half maximal proliferation of the B9 cells). The specificity of the assay was confirmed by pre-incubation of the samples with neutralizing antibodies against IL-1 $\alpha$ , IL-1 $\beta$  and IL-6.

#### Statistical analysis

Comparisons between the groups of patients were done using the Wilcoxon rank sum test. Student's *t*-test was used for statistical evaluation of bioassay data.

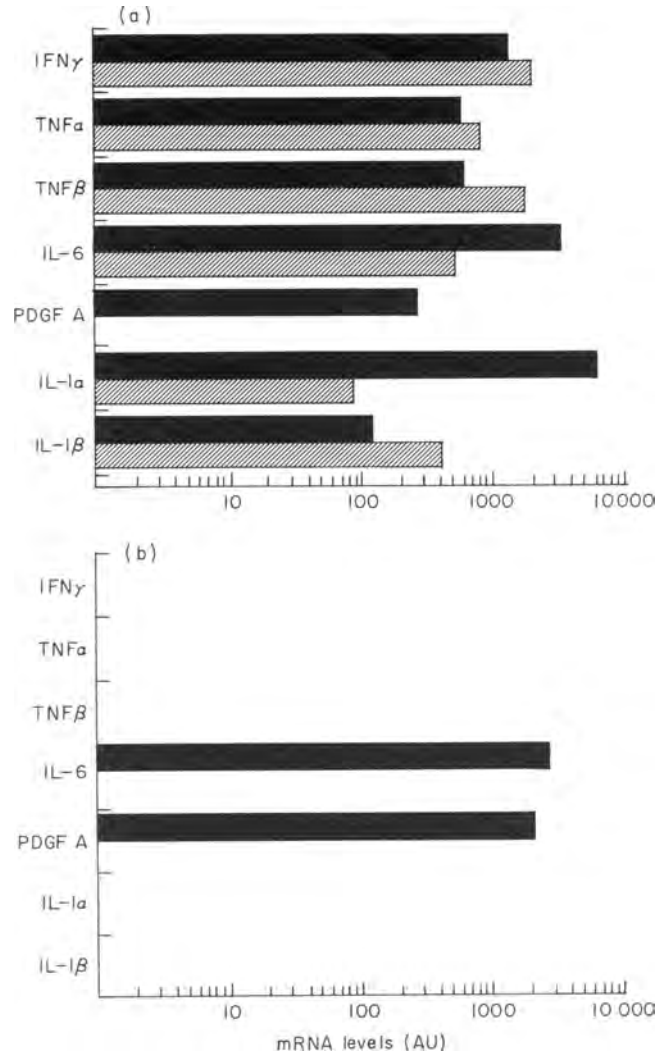
## RESULTS

#### Quantification of cytokine mRNA

Cytokine mRNA production in unpurified thyroid cells varied considerably within the groups (Fig. 1). Although values tended to be higher in GD than in non-toxic goitres (mean values were higher for each cytokine), non-producers were found in each group. In both groups cytokine production generally corresponded to the size of the intrathyroidal lymphocytic infiltrate. Typical examples of cytokine production in patients from both disease groups with large and small lymphocytic infiltrates is shown in Fig. 2. Surprisingly, the two HT samples which showed an extensive lymphocytic infiltrate, had relatively low IFN- $\gamma$  and TNF- $\alpha$  mRNA levels. It seems possible that at the stage of almost complete tissue destruction, which is typical for HT, lack of autoantigen stimulation results in the production of a different cytokine spectrum, in contrast with lymphocytes activated with polyclonal activators such as PHA and phorbol myristate acetate (PMA) which produce high levels of both TNF- $\alpha$ , IFN- $\gamma$  as well as TNF- $\beta$  mRNA (Buchan *et al.*, 1988a; Turner & Feldmann, 1988). IL-6 and PDGF A chain mRNA were found in almost all samples. Freshly purified TFC contained no mRNA for IFN- $\gamma$ , TNF- $\alpha$  or TNF- $\beta$  and only traces of IL-1 $\beta$  and PDGF-A message; however, high levels of IL-1 $\alpha$  mRNA were present in four out of eight GD and in one out of six non-toxic goitre samples, and IL-6 mRNA was present in TFC from four out of six GD and from two non-toxic goitre patients (data not shown).

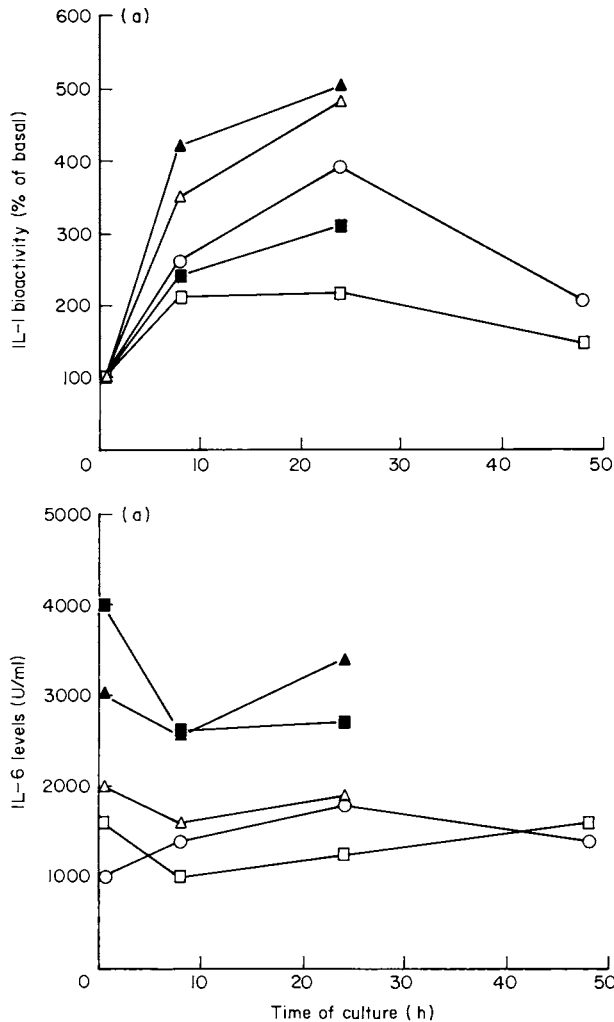
#### Release of cytokines by purified TFC

In order to determine whether TFC were capable of producing IL-1 $\alpha$  and IL-6 as was suggested from the mRNA data, IL-1



**Fig. 2.** Comparison of cytokine mRNA expression in patients with a large (a) and small (b) lymphocytic infiltrate. mRNA were extracted from unpurified thyroid cells from patients with either Grave's disease (■) or non-toxic goitre (■). The degree of lymphocytic infiltrate was determined semi-quantitatively by routine histological analysis as described in Materials and Methods. The data shows the levels of cytokine mRNA expression in arbitrary units (AU) in one patient from each group who was shown to have a large (a) or small (b) lymphocytic infiltrate.

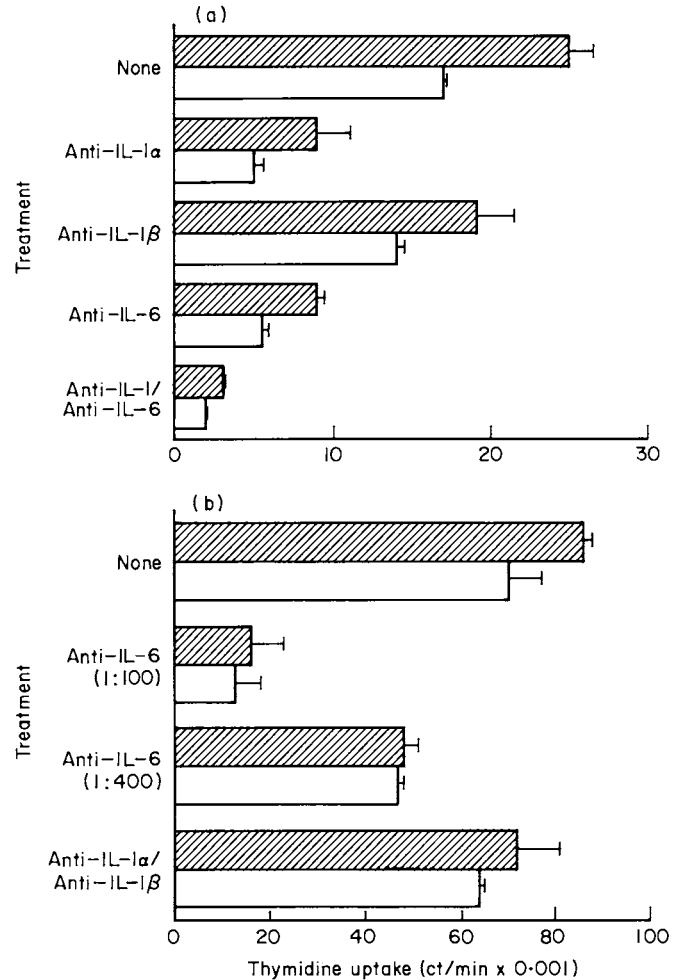
bioactivity was studied in TFC primary cultures, prior to and in response to LPS stimulation. Supernatants conditioned by thyroid follicular cells not only stimulated the IL-1/IL-6 dependent proliferation of mouse thymocytes in response to PHA (Fig. 3a), but also induced the growth of the IL-6-dependent hybridoma B9 (Fig. 3b). These stimulatory activities were found in the supernatants conditioned by non-toxic goitre as well as by GD TFC. Although thymocyte-stimulating activity was also found in unstimulated samples, its secretion could be considerably increased by addition of LPS to culture medium. In contrast, the stimulation of the B9 hybridoma by conditioned supernatants was not influenced by pretreatment of thyroid follicular cells with LPS, suggesting IL-1 production is readily



**Fig. 3.** IL-1 (a) and IL-6 (b) bioactivity in supernatants conditioned by LPS-stimulated TFC from patients with non-toxic goitre (NTG) or Graves' disease (GD). (a) Stimulation of PHA-induced mouse thymocyte proliferation. Values are expressed as percentage of PHA-induced  $^3\text{H}$ -thymidine uptake in thymocytes after addition of conditioned supernatants from LPS untreated TFC, which was considered as 100% (mean  $31950 \pm 12249$  s.e.m., range 18198–37233 ct/min; the response to PHA alone did not exceed 6000 ct/min). (b) Levels of IL-6 measured using the B9-IL-6 dependent hybridoma. Units/ml in each assay were normalized to the slightly varying TFC numbers in the different samples, cell numbers referring to the end of the culture period.  $\square$ , NTG 1;  $\blacksquare$ , NTG 2;  $\triangle$ , NTG 3;  $\blacktriangle$ , NTG 4; and  $\circ$ , GD.

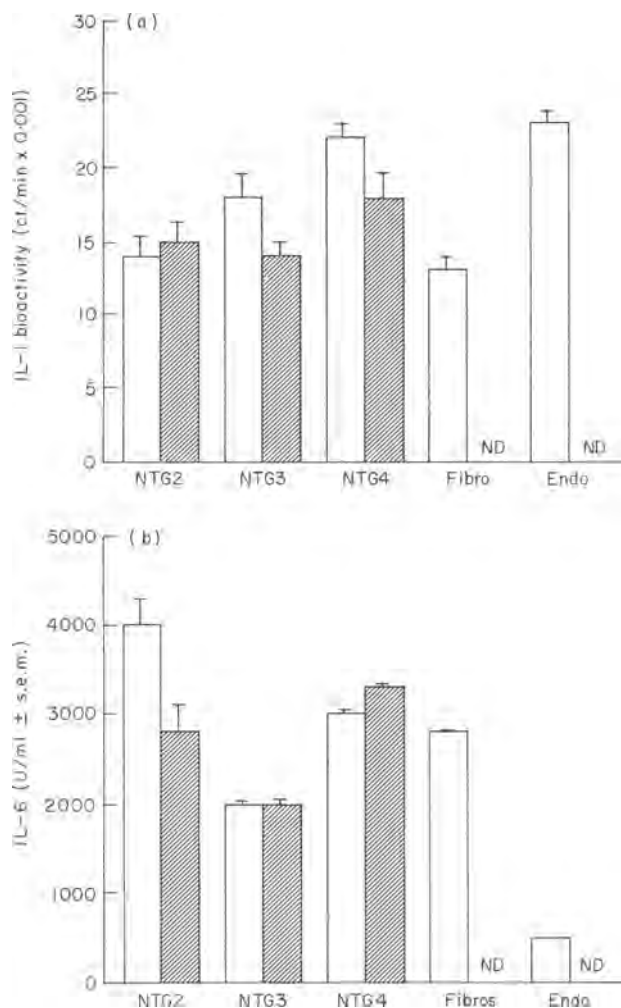
inducible by LPS while IL-6 may be produced constitutively by these cells.

Stimulation of thymocyte proliferation could be completely blocked by pre-incubation of the supernatants with a mixture of antibodies against IL-1 $\alpha$ , IL-1 $\beta$  and IL-6, whereas addition of a single antibody reduced the stimulatory effect by 50% in the case of anti IL-1 $\alpha$  and anti IL-6, but had only a minor effect in the case of anti IL-1 $\beta$  (Fig. 4a). In contrast, stimulation of B9 hybridoma cell growth could be suppressed by anti-IL-6, but not by either anti-IL-1 (Fig. 4b). These data confirmed the mRNA results in freshly purified cells, demonstrating that TFC can produce IL-1 $\alpha$  and IL-6.



**Fig. 4.** Blocking of IL-1 and IL-6 bioactivity in supernatants conditioned by LPS-stimulated TFC (24 h stimulation) with polyclonal antibodies specific for IL-1 $\alpha$ , IL-1 $\beta$  or IL-6. Proliferation is indicated as ct/min  $\pm$  s.e.m. from triplicate cultures. (a) Mouse thymocyte assay showing one GD ( $\square$ ), and one non-toxic goitre ( $\blacksquare$ ) patient. The response to PHA alone was  $3420 \pm 127$ . (b) Supernatants from GD ( $\square$ ) and non-toxic goitre ( $\blacksquare$ ) TFC were incubated with goat anti-IL-6 at 1:100 or 1:400 dilution or a mixture of rabbit anti-IL-1 $\alpha$  and IL-1 $\beta$  antibodies, IL-6 activity was determined using the B9 hybridoma cell assay as described in Material and Methods.

In order to define whether the IL-1 and IL-6 production by the TFC was not due to the 5% contaminating cells, not stained by antimicrobial antibody (macrophages, endothelial cells and fibroblasts), IL-1 and IL-6 bioactivity were re-investigated after complement killing of two of the three possible contaminating cell types. Depletion of contaminating endothelial cells and macrophages, which led to a 98–99% purity of the TFC, resulted in only a minor decrease in IL-1 and IL-6 production (Fig. 5). No complement-fixing antibody against fibroblasts was available. However, fibroblast contamination, as assessed by staining of cells with the F15-42-1, anti-human Thyl antibody, was  $< 2\%$  in all cases, and IL-1 and IL-6 production did not differ between samples with a relatively high (2%) and a relatively low (0.2%) content of fibroblasts (data not shown), suggesting that contaminating fibroblasts were not responsible for the IL-1 $\alpha$  and IL-6 bioactivity secreted. Consistent with this



**Fig. 5.** Comparison of IL-1 (a) and IL-6 (b) bioactivity in supernatants conditioned by TFC of 95% purity ( $\square$ ), and TFC of 98–99% purity after treatment with antibody and complement as described in Materials and Methods ( $\blacksquare$ ). IL-1 and IL-6 bioactivity released by an equal number of pure human endothelial cells (Endo) or pure human fibroblasts (Fibro) is shown for comparison. (a) Mouse thymocyte assay. (b) B9 hybridoma cell assay. Both assays were performed as described in Materials and Methods. NTG, non-toxic goitre; ND, not done.

suggestion, equal numbers of cultured human fibroblasts produced similar amounts of IL-1 and IL-6 as purified TFC (Fig. 4). A equal number of cultured pure human endothelial cells produced similar quantities of IL-1 and less IL-6 than thyroid follicular cells (Fig. 5). From these results it seems very unlikely that the IL-1 and IL-6 bioactivity found in supernatants from TFC primary cultures reflects the production by the 5% contaminating cells only, but strongly suggests that TFC can themselves produce these cytokines.

## DISCUSSION

We have developed a concept of endocrine autoimmunity in which HLA class II expressing epithelial target cells present autoantigens to autoreactive T cells (Bottazzo *et al.*, 1983). This has been experimentally tested, and it has been shown that HLA

class II expressing thyroid epithelial cells can present specific peptide antigens (Londei *et al.*, 1984) as well as restimulate autoreactive T cells (Londei, Bottazzo & Feldmann, 1985). However, while aberrant expression of HLA class II is not restricted to autoimmune diseases, self-reactive T cells appear to be a specific component of the disease (Grubeck-Loebenstein *et al.*, 1988). Soluble mediators, of which the best characterized is IFN- $\gamma$  induce the expression of HLA class II on thyroid epithelial cells *in vitro* (Todd *et al.*, 1985) and may synergize with other cytokines such as TNF- $\alpha$  (Kissonerghis *et al.*, 1989). However, it had not been determined whether IFN- $\gamma$  or TNF- $\alpha$  was expressed in thyroid tissue. Thus, the objective of this study was to characterize cytokine production in thyroid tissue during the disease process. The presence of lymphoid infiltrates in thyroid autoimmune (GD and HT) and non-autoimmune (non-toxic goitre) conditions provided an opportunity to compare cytokine gene expression in an autoimmune process and in non-autoimmune inflammation using thyroid surgical specimens. The techniques used were based on our work with active rheumatoid arthritis joint tissues, where we had found that mRNA assays were a quick and reliable way to establish which cytokine genes were activated (Buchan *et al.*, 1988a; b).

Using the slot-blotting procedure, we have found that the mediators investigated, IFN- $\gamma$ , TNF- $\alpha$ , TNF- $\beta$  (lymphotoxin), IL-1 $\alpha$ , IL-1, IL-6 and PDGF-A chain are present in the GD thyroid tissue. However, there were considerable differences between patients, which appeared to correlate with the degree of lymphoid infiltration. In the two HT patients there was little IFN- $\gamma$  and TNF- $\alpha$  mRNA; however, TNF- $\beta$  mRNA was expressed at high levels, suggesting a different pattern of cytokines may be produced in this disease (Fig. 1a). Cytokine production was also found in non-toxic goitre, although at a lower level than in GD. IL-6 and PDGF A chain mRNA were found in most of the samples, even in the ones with very little infiltrate. Unlike IFN- $\gamma$ , TNF- $\alpha$  and IL-1, there was little difference between GD and non-toxic goitre with respect to PDGF A chain and IL-6 mRNA content (Fig. 1). Very similar results were obtained using infiltrating mononuclear cells (Fig. 1b). The patterns of cytokine mRNA observed are not consistent with simple activation of mononuclear cell fraction during the isolation procedure, as HT samples, which had large mononuclear cell infiltrates, contained little mRNA for TNF- $\alpha$  but large amounts of TNF- $\beta$  mRNA. The TNF- $\alpha$  gene is very sensitive to activation by agents such as LPS (Beutler & Cerami, 1988); thus, our failure to detect its RNA suggests that the isolation procedures do not promote cell activation. It is perhaps not too surprising that the results of the autoimmune diseases overlapped with those of non-toxic goitre, since this disease, while not autoimmune, has an inflammatory component and often shows HLA class II expression and the development of antithyroid antibodies (Grubeck-Loebenstein *et al.*, 1986). Elsewhere we have demonstrated that non-toxic goitre is characterized by a lack of transforming growth factor (TGF $\beta$ ), an inhibitor of TFC growth (Grubeck-Loebenstein *et al.*, 1989). As TGF $\beta$  has potent immunosuppressive activities (Sporn & Roberts, 1988) it is possible that the lack of this immunosuppressive entity is the reason for the inflammatory response frequently noted in non-toxic goitre. The studies reported here reveal that cytokine gene expression is very heterogeneous in thyroid surgical specimens of the three diseases studied.

IL-1 $\alpha$  and IL-6 mRNA were found at high levels in the purified TFC preparation. In addition, media conditioned by these cells contained the appropriate bioactivity (Figs 2–4). Stimulation of thymocyte proliferation by conditioned supernatants from TFC has previously been reported (Hirose *et al.*, 1987), but the nature of the activity was not defined. Our results using highly specific antibodies raised to recombinant proteins show that thymocyte stimulation is due to the presence of IL-6 as well as of IL-1 $\alpha$ , but that the contribution of IL-1 $\beta$  is minimal. The production of IL-6 was further shown by the stimulatory effect of conditioned supernatants on the growth of the IL-6 dependent hybridoma B9. This was specifically inhibited by anti-IL-6 antisera. TFC are adherent and thus certain adherent cells may contaminate the preparation, such as endothelium, fibroblasts and macrophages. The proportion of contaminating cells was 5%, and treatment of TFC with monoclonal antibodies and complement against macrophages and endothelial cells reduced levels to 1 or 2% without changing IL-1 $\alpha$  and IL-6 production.

These results reported here establish that TFC are, like many other cells (reviewed by Oppenheim *et al.*, 1986), capable of producing IL-1 $\alpha$  and IL-6; this observation may be of importance for the activation of autoreactive T cells, as clear evidence has recently emerged that these cytokines are costimulators of T cell activation. Recent work has shown that the combination of IL-1 and IL-6 can restore the capacity of accessory cell-depleted T cells to respond to PHA or anti CD3 antibodies (Uyttenhove, Coulie & Van Snick, 1988; Houssiau *et al.*, 1988), a property that neither cytokine alone possesses; it is believed IL-1 and IL-6 represent the major 'accessory factors' present in macrophage-conditioned medium. Recently, IL-6 was shown to augment T cell proliferation in response to anti CD28 antibodies (Baroja *et al.*, 1988). Several investigators have also suggested that IL-6 may act primarily during the early stages of T cell activation, as the IL-6 receptor (unlike the IL-1 or IL-2 receptor) is expressed at its highest density on small resting T cells and its levels decline following PHA stimulation (Taga *et al.*, 1987). If IL-1 $\alpha$  and IL-6 released by target cells are important for the restimulation of autoreactive T cells, this should be experimentally verifiable by *in vitro* by co-culture of thyroid autoreactive T cells and TFC in the presence of antibodies to IL-1 $\alpha$  and IL-6 using the protocols described previously (Londei *et al.*, 1985).

The production of IL-1 and IL-6 (this study) as well as TGF $\beta$  (Grubeck-Loebenstein *et al.*, 1989) by TFC emphasize that cytokine production is not restricted to mononuclear cells in autoimmune disease. We have provided evidence that the target tissue produces a number of cytokines that influence immune cells and these may contribute actively to the disease process. The full extent of this bi-directional communication remains to be established.

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