Clin. exp. Immunol. (1992) **90**, 522–529

Detection of cytokines at the site of tuberculin-induced delayed-type hypersensitivity in man

C. Q. CHU, M. FIELD, E. ANDREW, D. HASKARD*, M. FELDMANN† & R. N. MAINI *Division of Clinical Immunology, Kennedy Institute of Rheumatology, *Department of Rheumatology, Hammersmith Hospital, and †Kennedy Institute of Rheumatology at the Sunley Research Centre, London, UK*

(Accepted for publication 13 August 1992)

SUMMARY

Cytokines are chiefly local mediators which play an important role in the regulation of the cell–cell interactions which may be involved in the development of the delayed-type hypersensitivity (DTH) reaction. Using immunohistochemical techniques, the presence of IL-1α, IL-1β, IL-6, interferon-gamma (IFN-γ) and tumour necrosis factor-alpha (TNF-α) in the skin in tuberculin-purified protein derivative (PPD)-induced DTH reactions was investigated in six normal individuals. Cells staining for these cytokines were first observed 6 h after PPD challenge, and they were detected throughout the duration of the 7-day experiment. The number of cells staining for IFN-γ reached a peak at 48 h, where 33% of the total aggregate cells were positive, but declined thereafter to 3% at day 7. On the other hand, the number of cells staining for TNF-α and IL-1 persisted at high levels throughout the observation period of 7 days (e.g. at 48 h and thereafter, about 40% cells positive for TNF-α and 20% for IL-1α and IL-1β). Double immunofluorescence and staining on sequential sections showed that IFN-γ-staining cells were $CD3^+$ T cells; TNF-α, IL-1 and IL-6 staining cells were mainly of the $CD68^+$ macrophages/monocytes and that 80% of the $CD1a^+$ cells (Langerhans-like cells) in the dermis contained TNF-α and IL-1. The presence of these cytokines at the site of inflammation suggests that they may be locally produced by the inflammatory cells. Their persistence during the reaction suggests that they are intimately associated with this response, and are involved in the development of the reaction.

Keywords IL-1 IL-6 tumour necrosis factor-alpha interferon-gamma tuberculin-induced delayed-type hypersensitivity

INTRODUCTION

The cutaneous tuberculin reaction is a classical example of delayed-type hypersensitivity (DTH) in man. The cellular components involved in this response have been well documented, in which T lymphocytes, macrophages and Langerhans cells are recruited into the site of the lesion [1–5]. The tuberculin skin test provides a useful *in vivo* model for studying the effector arms of the immune response, since the response occurs in otherwise normal skin. The kinetics of the response can be effectively monitored as the time of antigen application is known, whereas this is not usually known in disease.

Cytokines, particularly those produced by T cells and monocyte/macrophages, are believed to play an important role in the regulation of cell-mediated immune and inflammatory responses. It is thought that T cells are activated by an antigen and accumulate at the site of the antigen, releasing lymphokines which induce the activation and proliferation of the monocytes/macrophages. The monocytes/macrophages in turn release monokines which regulate the function of T cells and other cell types. Thus understanding the expression of cytokines *in vivo* in an induced model of DTH reaction should provide useful information about the role of these molecules in cell-mediated immune and inflammatory responses in general. To date, there are only limited data on local expression of cytokines during the human tuberculin DTH response, in which IL-2 has been demonstrated at the site of skin lesion in a tuberculin-purified protein derivative (PPD)-induced DTH reaction in patients with pulmonary tuberculosis [6]. A recent study using *in situ* hybridization demonstrated the preferential expression of mRNA for IL-2 and interferon-gamma (IFN-γ) by infiltrating T cells at the site of lesion in PPD-induced DTH reaction in normal individuals [7]. However, little attention has been paid to the expression of monocyte/macrophages-derived cytokines in this response.

The aim of this study, using immunohistochemical techniques, was to investigate the local expression of IL-1α, IL-1β,

Correspondence: Dr C. Q. Chu, Division of Clinical Immunology, Kennedy Institute of Rheumatology, 6 Bute Gardens, Hammersmith, London W6 7DW, UK.

IL-6, tumour necrosis factor-alpha (TNF-α) and IFN-γ at the site of skin lesion in tuberculin DTH reaction in normal individuals, and to document the dynamics of the expression during this response.

MATERIALS AND METHODS

PPD immunization and tissue processing

Six bacille Calmette–Guerin (BCG)-immunized volunteers were included in this study. Ten units (0·1 ml of 100 U/ml solution) of PPD (Evans Medical Ltd, Horsham, UK) were injected intradermally into the forearm. The reactions were observed and the induration at each time point was measured before the biopsy was taken. A variable number (3–6) of 4 mm full-thickness skin punch biopsies were taken at 6, 24, 48, 72 h and 7 days after the PPD injection. Two of the volunteers donated four biopsies at 6, 24, 48 h and day 7, and at 6, 24, 72 h and day 7 respectively. Biopsies of normal skin were also taken before the PPD injection. The fresh tissue was snap-frozen in isopentane cooled in a bath of liquid nitrogen and stored at −70°C. Five-micrometre sections were cut on a cryostat and placed onto poly-L-lysine-coated slides.

Immunohistochemical staining

The sections were routinely stained with haematoxylin and eosin to examine the histological changes during the reaction. Mouse cell marker MoAbs (Table 1) were used to analyse the cellular components of the PPD-induced DTH reaction. MoAb L243 (Table 1) was used to detect HLA-DR expression. The sections were fixed and blocked as described previously [8], and the antibodies were incubated with the sections for 16 h at 4°C. The sections were washed in 0·05 M Tris-buffered saline (TBS) pH 7·36, followed by incubation with goat anti-mouse immunoglobulins diluted 1:20 (Dakopatts, Glostrup, Denmark) for 60 min at room temperature. The sections were washed again and incubated for 60 min with alkaline phosphatase-conjugated mouse anti-alkaline phosphatase complex (APAAP, Dakopatts) diluted 1:50. The staining was developed with 1 mg/ml Fast Red (4-chloro-2-methylbenzenediazonium zinc chloride, Sigma Chemical Co., Poole, UK) in TBS pH 8·2 containing 0·6 M dimethyl-formamide, 0·02 M naphthol phosphate and 0·001 M levamisole.

In addition, B lymphocytes/plasma cells were also assessed by direct immunofluorescence staining using FITC-conjugated goat anti-human $F(ab')_2$ (ICN, Lisle, IL).

The expression of cytokines in the DTH skin reaction was detected using immunohistochemical techniques as described previously [8]. Briefly, fixed and blocked tissue sections were incubated for 90–120 min with previously characterized biotinylated rabbit IgG $F(ab')_2$ fragments (Table 1) against human IL-1α, IL-1β, IL-6, TNF-α and IFN-γ. The binding was detected by incubation of the sections with streptavidin-linked alkaline phosphatase (Amersham, Aylesbury, UK) diluted 1:50 in 2% normal goat serum in TBS for 60 min. Colour was developed with Fast Red as above and the slides mounted. Biotinylated normal $F(ab')_2$ fragments and cytokine-adsorbed anti-cytokine antibodies were included as negative control antibodies in the immunohistochemical staining [8].

In order to characterize the cytokine-containing cells, sequential section staining was performed with anti-cytokine and EBM11 (CD68) and Leu-4 (CD3) antibodies. Double immunofluorescence staining [8] was also performed on same sections with combination of anti-cytokine and EBM11, Leu-4, BL6 (CD1a) and L404 (CD1a) antibodies (Table 1). The binding of cell marker MoAbs was detected with FITC-conjugated goat anti-mouse IgG (Sigma). The biotinylated anti-TNF-α, IL-6, IL-1α and IL-1β $F(ab')_2$ fragments were developed with streptavidin-linked Texas Red (Amersham).

Cell quantification

The numbers of cells stained with cell marker antibodies and cytokine-positive cells were counted. In normal skin and biopsies taken at 6 h, at least total 500 cells in the dermis were counted in each section. In the samples taken at 24 h and thereafter, only cells within cell aggregates in the dermis were counted and at least five aggregates were included in each section. Average percentages of positive cells over total cells were taken from duplicates of each sample. In order to compare

Table 1. Primary antibodies used in the study

Antibody	Specificity	Isotype	Dilution/protein concentration	Source (reference)
Leu-4	Pan T cells (anti-CD3)	Mouse IgG1	1:5	Becton Dickinson, Mountain View, CA
ABL4	Inducer/helper T cells (CD4)	Mouse IgG2a	Ascites 1:100	Dr J. Brochier, INSERM U291, Montpellier, France [37]
BL15	Suppressor/cytotoxic T cells (CD8)	Mouse IgG2b	Ascites 1:100	Dr J. Brochier [37]
BL6	Langerhans cells (CD1a)	Mouse IgG1	Ascites 1:100	Dr J. Brochier [37]
L404	Langerhans cells (CD1a)	Mouse IgG1	Ascites 1:100	Dr J. Brochier [37]
EBM11	Macrophages/monocytes (anti-CD68)	Mouse IgG1	3 μg/ml	Dakopatts, Glostrup, Denmark
L243	HLA-DR	Mouse IgG2a	Ascites 1:200	ATCC, Rockville, MD
L26	B cells (CD20)	Mouse IgG2a	1·5 μg/ml	Dakopatts
Anti-Hu-Ig $F(ab')_2$	B/plasma cells	Goat immunoglobulin $F(ab')_2$-FITC	1:50	ICN, Lisle, IL
Anti-IL-1α	IL-1α	Rabbit IgG $F(ab')_2$-biotin	10 μg/ml	[38]
Anti-IL-1β	IL-1β	Rabbit IgG $F(ab')_2$-biotin	10 μg/ml	[38]
Anti-IL-6	IL-6	Rabbit IgG $F(ab')_2$-biotin	15 μg/ml	[38,39]
Anti-TNF-α	TNF-α	Rabbit IgG $F(ab')_2$-biotin	10 μg/ml	[7,38]
Anti-IFN-γ	IFN-γ	Rabbit IgG $F(ab')_2$-biotin	20 μg/ml	[38,40]

the number of T cells and of macrophage/monocytes in the dermis, the percentages of $CD3^+$ and $CD68^+$ cells over total cells were determined at each time point. The percentage of Langerhans cells in the epidermis was expressed as the number of BL6/L404 antibody-staining cells per 100 basal cells. Langerhans cells staining for cytokines were expressed as cytokine-containing cells over $BL6/L404^+$ cells.

RESULTS

Clinical and histological appearance of the DTH reaction

All the volunteers receiving PPD reacted with a red induration and swelling at the site of injection. Six hours after PPD injection, increased numbers of cells in the dermis were observed. An intensive dermal infiltration of mononuclear cells with cell aggregate formation was seen 48 h after PPD injection (Fig. 1), and this persisted throughout the observation period of 7 days.

Cellular phenotypes in the PPD-induced DTH reaction

T lymphocytes. Less than 3% $CD3^+$ T cells were seen scattered in the dermis in normal skin. Six hours after the injection of PPD, an increased number of T cells were seen scattered in the sub-epidermal area, around blood vessels, with some in clusters. The number of T cells increased markedly at 24 h and reached a peak at 48 h, but declined at 72 h and then remained constant until day 7 (Fig. 2a). A few $CD4^+$ T cells were found in the epidermis at 48 and 72 h. The CD4:CD8 T cell ratio remained approximately 2:1 throughout the reaction.

Macrophages. Cells staining with anti-CD68 antibody were sparsely distributed in the dermis in normal skin. At 6 h, a slightly increased number of macrophages was detected around blood vessels and some in small clusters. The number of macrophages rapidly increased from 24 to 48 h and reached a peak at 72 h. A slight decrease in the number of these cells was seen at day 7 (Fig. 3a). Throughout the reaction, the macrophages were mainly localized in the cell aggregates interspersed between the T cells. Some were scattered between the cell aggregates.

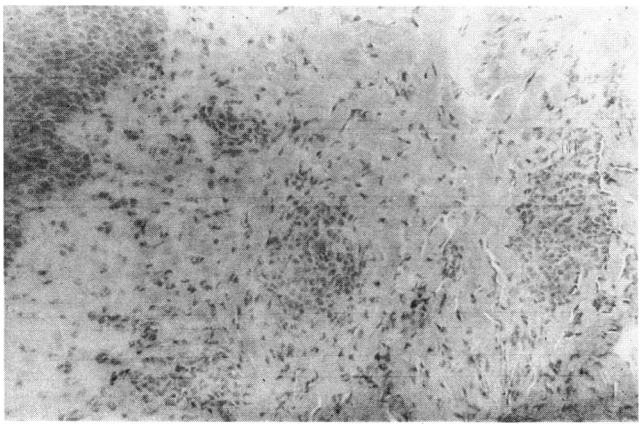
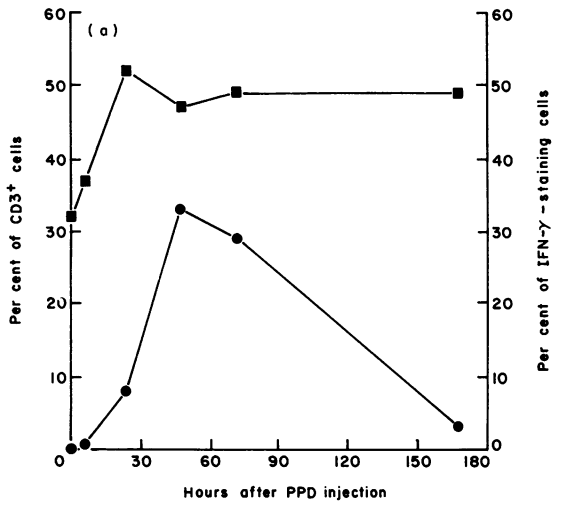


Fig. 1. Skin biopsy taken at 48 h after the injection of PPD shows intensive infiltration of mononuclear cells with formation of cell aggregates in the dermis (haematoxylin and eosin staining, × 32).



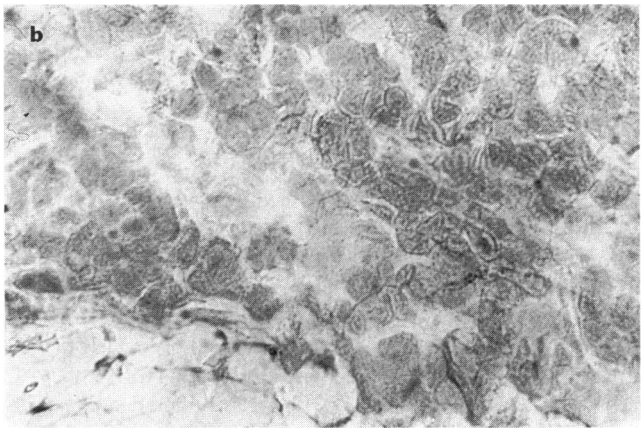


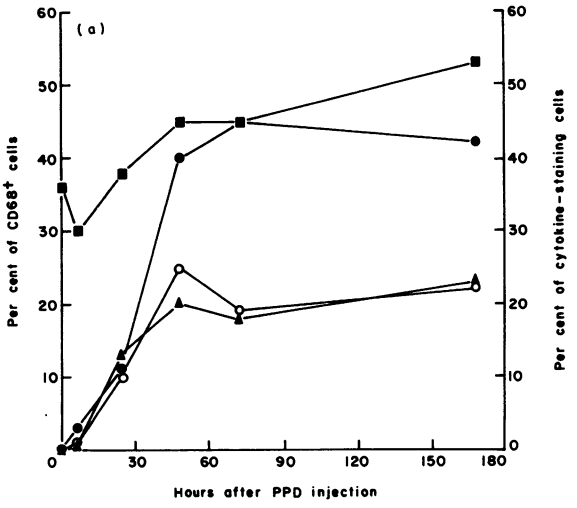
Fig. 2. (a) Kinetics of IFN-γ staining cells in the PPD-induced DTH reaction. The number of cells staining for IFN-γ in the dermis increased with time of reaction and reached a peak at 48 h, declined at 72 h and further down at day 7. ■, $CD3^+$ cells; ●, IFN-γ. (b) A skin sample taken at day 7 shows few cells stained for IFN-γ and the staining intensity was low (immunohistochemical staining with alkaline phosphatase and counterstained with haematoxylin, × 160).

Langerhans cells. Langerhans cells were defined by MoAbs BL6 and L404 which both recognize the CD1a antigen. In normal skin 3–5 per 100 basal cells Langerhans cells were observed in the epidermis. The number of Langerhans cells increased at 24 h in the epidermis, reached a peak of about two-fold the number in normal epidermis at 48 h, and declined thereafter. Langerhans cells were also seen in the upper dermis and some in cell aggregates at 48 and 72 h, but not at other time points.

B lymphocytes. No cells staining with FITC-conjugated $F(ab')_2$ fragments of goat anti-human immunoglobulin $F(ab')_2$ antibodies were observed in the skin sections throughout the reaction of experimental period. No cells were found stained with anti-CD20 antibody.

Expression of HLA-DR

In the dermis in skin biopsies taken before the injection of PPD, only a few cells were seen to stain for HLA-DR. The number of cells staining for HLA-DR increased from 6 h after PPD injection, and at 24 h and thereafter almost 90% of the



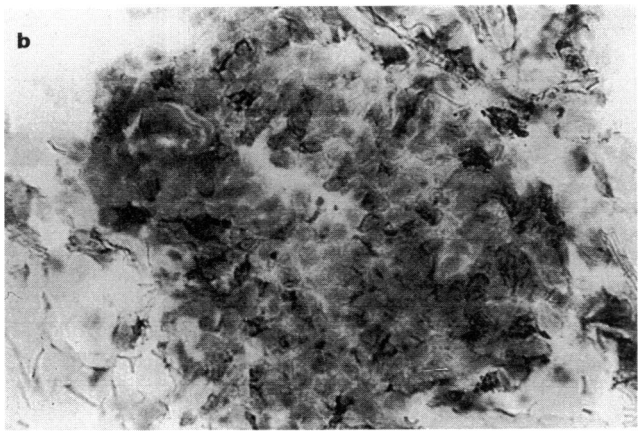


Fig. 3. (a) Kinetics of IL-1 and TNF-α staining cells in the PPD-induced DTH reaction. The numbers of cells staining for IL-1α, IL-1β and TNF-α persisted at high levels until at day 7. ■, $CD68^+$ cells; ●, TNF-α; ▲, IL-1α; ○, IL-1β. (b) Immunohistochemical staining of skin taken at day 7 shows many cells strongly stained for TNF-α (immunohistochemical staining with alkaline phosphatase and counterstained with haematoxylin, × 80).

infiltrating cells stained positively for HLA-DR. Some cells with dendritic morphology in both dermis and epidermis at 48 and 72 h were stained. In addition, some of the keratinocytes also stained for HLA-DR.

Local detection of cytokines in the PPD-induced DTH reaction

Detection of cytokines in normal skin. A few cells (0·5–0·7%) in the normal skin sections stained for TNF-α, IL-6 and IL-1α in the epidermis. The staining appeared to be associated with keratinocytes. None of the cells in the dermis was found to stain with any of the anti-cytokine antibodies.

Detection of cytokines after PPD injection. As shown in Table 2, all of the cytokines examined (IL-1α, IL-1β, TNF-α, IL-6 and IFN-γ) were readily detected in the PPD-induced DTH reaction. Cells staining for the cytokines were mainly found within cell aggregates (Fig. 4). Staining of sequential sections and double immunofluorescence staining on same sections showed that IFN-γ-staining cells were located in the area of T cells (Fig. 5a,b) in the dermis, and IL-1, IL-6 and TNF-α-staining cells were found in the area of the macrophages (Fig. 5c, d). At 48 and 72 h, some of the T cells infiltrating the epidermis showed staining for IFN-γ. Double immunofluorescence staining showed 80% of the Langerhans cells contained TNF-α, IL-6, IL-1α, and IL-1β in both the epidermis and dermis (Fig. 5e, f), suggesting that these cells produced the cytokines during the reaction. Around 2–5% of the epidermal cells were found to be stained with antibodies to IL-1α, IL-6 and TNF-α during the reaction. Neither the normal rabbit $F(ab')_2$ fragments nor the cytokine-adsorbed anti-cytokine antibodies showed any staining in the sections.

Kinetics of cytokine-containing cells. The kinetics of IFN-γ, TNF-α, IL-1α and IL-1β staining cells were analysed in two volunteers. Cells staining for IFN-γ were first seen in the upper dermis 6 h after PPD injection. At 24 h and beyond the IFN-γ-positive cells were mostly found in the cell aggregates. The number of IFN-γ-positive cells increased with time and reached a peak at 48 h where 33% of the total cells in aggregates were positive, but declined at 72 h. The number of IFN-γ-positive cells further decreased to 3% at day 7 and the staining intensity was much lower (Fig. 2).

Table 2. Percentage of cytokine-containing cells detected in the dermis in PPD-induced DTH reaction*

	Time after PPD injection (h)					
Cytokine (%)	0 ($n=6$)†	6 ($n=3$)	24 ($n=4$)	48 ($n=6$)	72 ($n=4$)	168 ($n=3$)
IL-1α	0	1 (0·7–1·2)	10 (6–15)	25 (20–30)	19 (15–24)	22 (20–24)
IL-1β	0	0·5 (0·2–0·7)	13 (7–17)	20 (15–30)	18 (12–22)	23 (20–26)
IL-6	0	ND‡	ND	30 (21–39)	35 (30–38)	29 (21–33)
TNF-α	0	3 (1·5–6)	11 (7–16)	40 (29–47)	45 (39–54)	42 (29–45)
IFN-γ	0	0·5 (0·3–0·8)	8 (4–12)	33 (20–41)	29 (19–33)	3 (1–5)

* In all the skin sections, cells in sweat glands and hair follicles were not counted and at 24 h and thereafter, only the cells in cell aggregates were counted. Average percentages and ranges of cytokine-containing cells are shown.

† Number of skin biopsies examined.

‡ ND, Not done.

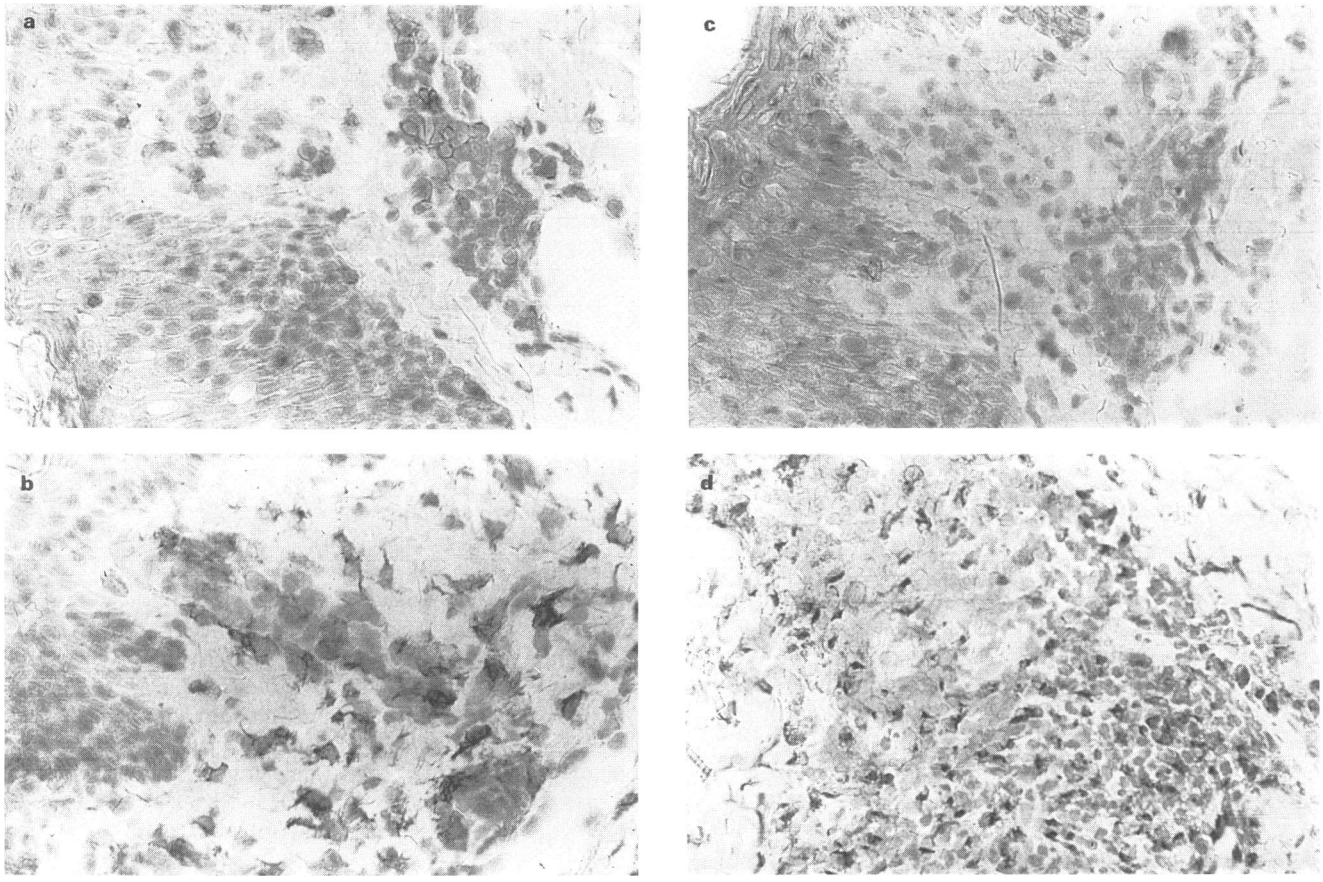


Fig. 4. Immunohistochemical staining with alkaline phosphatase of skin biopsies of the PPD-induced DTH reaction at 48 h shows: (a) IFN-γ and (b) IL-1α staining cells at upper dermis; (c) biotinylated normal rabbit F(ab')$_2$ fragments did not stain any cells; and (d) TNF-α staining cells in a cell aggregate at deep dermis. (Sections were counterstained with haematoxylin, $\times$80).

The appearance of cells staining for IL-1α and IL-1β and TNF-α all had similar kinetics (Fig. 3). The positive cells were first seen in the upper dermis 6 h after PPD injection. The number of positive cells increased throughout the reaction and were still present in a higher proportion of cells at day 7 (Table 1). The numbers of IL-1-staining cells reached a peak at 48 h (25% IL-1α; 20% IL-1β) and slightly declined thereafter (Fig. 3). A peak of TNF-α-staining cells was observed at 72 h where 45% of the cells were positive, and this persisted until day 7 after PPD injection (Fig. 3).

DISCUSSION

In this study, a range of cytokines were detected *in vivo* during a PPD-induced DTH reaction in man. In the dermis of the reactive skin, these cytokines were found mainly in the infiltrating lymphocyte and macrophage areas. The presence of these cytokines at the site of inflammation and their persistence during the reaction, in contrast to their absence in normal skin, suggests that they may be locally produced by inflammatory cells and are associated with this response, and possibly play an important role in the development of the reaction.

As demonstrated in the present study and by others [1,6], the major changes of the tuberculin DTH reaction occur in the dermis, and are histologically characterized by massive infiltration of mononuclear cells and the formation of cell aggregates. The mechanism of recruitment of the circulating leucocytes into the site where the antigen is present is not fully understood, but the local release of cytokines may play an important role. Cytokines can be involved in several aspects in the process of this reaction. First, cytokines such as IL-1 [9] and TNF-α [10] may act as chemotactic factors attracting leucocytes to the site of inflammation. The presence of these cytokines at the site of DTH reaction suggests they may be involved in recruiting immune cells during the response. Upon activation by an antigen, T cells and macrophages produce cytokines, recruiting more T cells and macrophages to the site of inflammation to amplify the response. IL-8 (not included in this study), a recently identified member of a family of low molecular weight cytokines, has a potent chemotactic effect on T cells [11]. A considerable amount of IL-8 has been detected in keratinocytes isolated from the skin during DTH reactions, and its production is considerably enhanced by IL-1 and TNF-α [12]. This suggests that IL-1 and TNF-α may exert their chemotactic effects indirectly via induction of IL-8 or other members of its family such as RANTES which is involved in the recruitment of CD45RO cells and monocytes [13].

Second, cytokines are also required in the proliferation and activation of immune cells and hence regulate the DTH reaction. For example, IL-2, a T cell-derived cytokine previously found in PPD-induced DTH reactions [6], is required for the proliferation of antigen-specific T effector cells [14]. IL-2 is

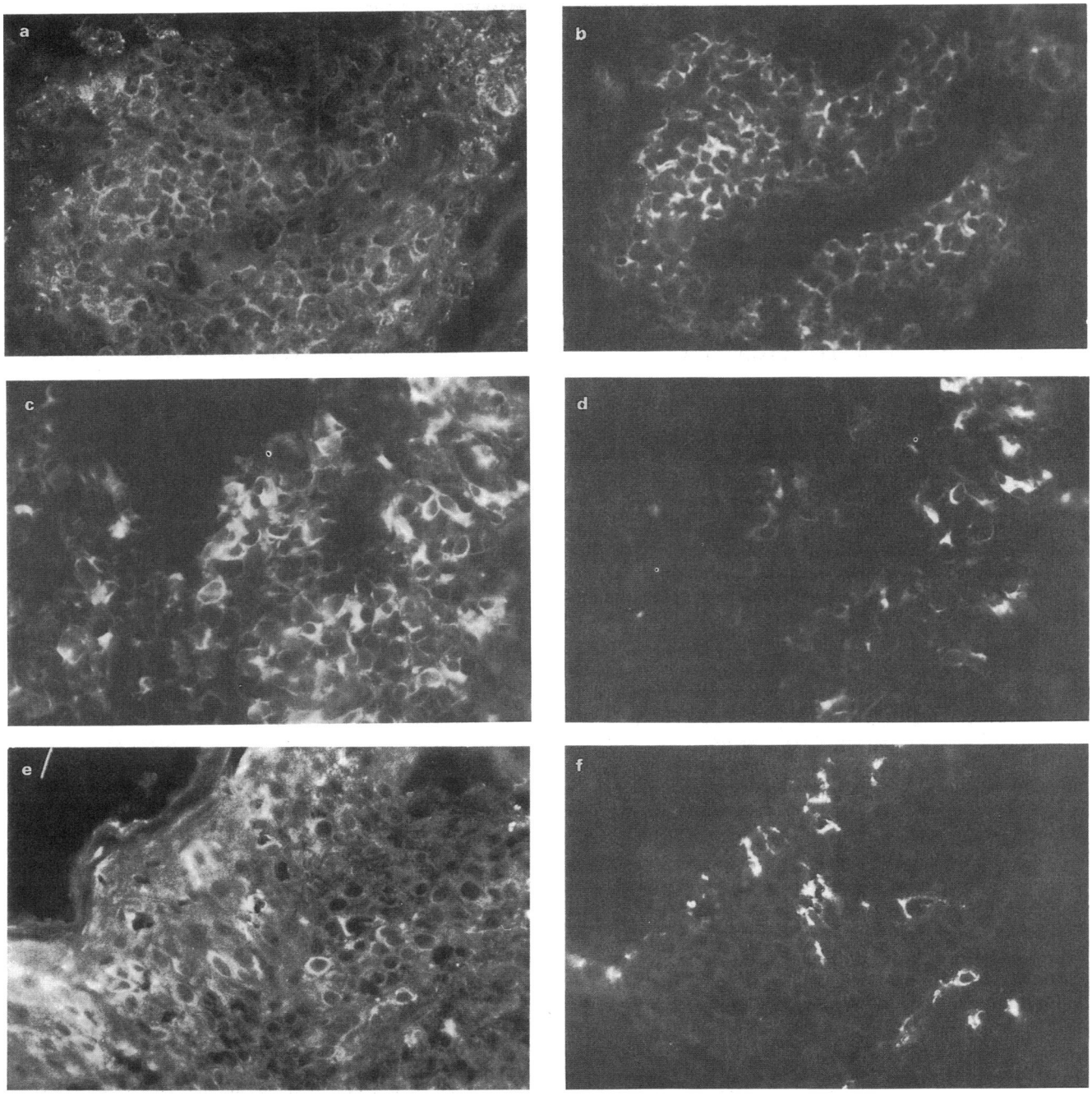


Fig. 5. Double immunofluorescence of skin sections taken at 48 h showing cytokine-containing cell phenotypes. (a) Cells staining for IFN-γ stained with (b) anti-CD3 antibody; (c) cells staining for TNF-α stained with (d) anti-CD68 antibody; (e) some TNF-α-staining cells also stained with (f) anti-CD1a antibody, suggesting production by Langerhans cells ($\times$ 160).

also a potent inducer of IFN-γ production [15], and IFN-γ activates macrophages and enhances their functions [16]. In addition, IL-1, IL-6 and TNF-α have all been shown to be involved in the activation and proliferation of T cells [17, 18].

Furthermore, cytokines regulate the expression of cell surface molecules which are important in cell–cell interactions. As demonstrated in the present and previous studies [1,6], from 24 h after PPD injection virtually all cells in the infiltrates are HLA-DR positive. It has been suggested that this expression may be a putative marker for IFN-γ production [6], because IFN-γ is known to be the most potent inducer of class II expression among the known cytokines. Indeed, a large number (up to 33%) of cells were shown to contain IFN-γ after PPD injection. Expression of MHC class II on the cell surface is known to be necessary for antigen presentation to T cells [19]. Thus by up-regulating MHC class II expression on antigen-presenting cells, cytokines up-regulate antigen presentation function and thereby can influence the immune response of T cells.

Cytokines are known to regulate the expression of cell adhesion molecules which are associated with leucocyte migration and homing in human immune responses and inflammation

(reviewed in [20]). IFN-γ has been shown to increase the binding of T cells to endothelial cells [21], and to be a major cytokine responsible for T cell migration [22]. Issekutz *et al.* [23] have demonstrated that *in vivo* IFN-γ stimulates T cells emigrating out of the blood into the site of DTH reaction. This effect of IFN-γ may be at least in part due to stimulation of intercellular adhesion molecule (ICAM)-1 expression on endothelial cells (EC) [24,25] and increased T cell binding to the EC through their cell surface lymphocyte function-associated antigen (LFA)-1 [21]. Both *in vitro* and *in vivo* studies have shown that TNF-α can enhance the endothelial expression of ICAM-1 induced by IFN-γ [24,26]. The cytokine-induced expression of ICAM-1 on EC is also involved in the binding of monocytes to EC [24]. Furthermore, the other vascular endothelium-associated adhesion molecules, vascular cell adhesion molecule (VCAM)-1, and E-selectin (endothelial leucocyte adhesion molecule (ELAM)-1) have also been shown to be expressed during the PPD-induced DTH reaction [27]. The expression of these molecules is also likely to be the result of local production of cytokines, particularly IL-1 and TNF-α [28].

The cytokines detected in the dermis were localized in cell aggregates, suggesting that they derive from infiltrating cells. In the study of the serial section and double staining, IFN-γ-staining cells were found, as expected, in the area of $CD3^+$ T cells. In fact, cloned murine helper T cells from DTH reactions are capable of secreting IFN-γ [29]. The other cytokines, TNF-α, IL-1, IL-6, are chiefly macrophage products and cells staining for these cytokines were in the area of $CD68^+$ cells, suggesting they were from cells of the macrophage/monocyte lineage.

TNF-α, IL-1 and IL-6 were also detected in the epidermis, suggesting that cells here were also producing cytokines during the reaction. The skin is currently recognized as an immunological active organ, as both epidermal and dermal cells may be involved in the initiation, amplification and regulation of immune response and inflammation [30]. Keratinocytes are known to be able to produce and secrete a large number of cytokines such as IL-1, IL-3, IL-6, IL-8, TNF-α, transforming growth factor (TGF) α and β and GM-CSF (reviewed in [31]). The present study has confirmed that *in vivo* a number of cytokines can be detected in keratinocytes, in both the normal condition and during the immune response to PPD. The production of cytokines by the keratinocytes may also contribute to PPD-induced DTH.

Langerhans cells expressing class II molecules are known to be antigen-presenting cells in the skin [32]. The number of Langerhans cells in the epidermis increased in the DTH reaction, and they were present at the site of lesions. It has also been demonstrated that Langerhans cells can produce cytokines such as IL-1 and TNF-α [33–35]. Using double immunofluorescence staining, 80% of the $CD1a^+$ Langerhans-like cells were found to also stain for TNF-α, IL-1α and IL-1β, both in dermis and epidermis during the reaction, suggesting production of these cytokines by the Langerhans cells.

It is noteworthy that the number of IFN-γ-staining cells declined after the peak at 48 h, and reduced to a small proportion at day 7. In contrast, the large number of cells staining for IL-1 and TNF-α persisted throughout the period of the 7-day observation. This suggests that IFN-γ production is transient, but IL-1 and TNF-α production tends to be more chronic. It is unlikely that the reduction of IFN-γ is due to the disappearance of T cells. At day 7 of the PPD-induced DTH reaction, although clinically the reaction is diminishing and the absolute number of infiltrating cells decreasing, a significant number of T cells (about 50%) and macrophages still persist in the lesion.

The results of the present study provide a model of the production of these cytokines during cell-mediated inflammation. The spectrum of cytokines in the PPD-induced DTH reaction was similar to that found in rheumatoid synovium [36], except that the T cell-derived cytokine IFN-γ was also readily but transiently detected in many cells in the PPD-induced DTH reaction but is not detectable in the rheumatoid arthritis (RA) synovial tissue [36]. However, this study is of a monophasic response which is more limited in duration than RA, thus the results do not reflect the cytokine production in a prolonged reaction such as in RA in which repeated or persistent exposure of the same antigen is possibly involved. In spite of this, these data may reflect the production of cytokines in the early stage of a chronic type inflammation and emphasize the early decay from the site of inflammation of the only T cell cytokine (IFN-γ) that we examined.

ACKNOWLEDGMENTS

This work was supported by the Arthritis and Rheumatism Council and by a grant from the Nuffield Foundation. C.Q.C. is supported by the Sino-British Friendship Scholarship Scheme and the Arthritis and Rheumatism Council.

REFERENCES

1 Poulter LW, Seymour GJ, Duke O, Janossy G, Panayi G. Immunohistological analysis of delayed-type hypersensitivity in man. Cell Immunol 1982; **74**:358–69.

2 Scheynius A, Klareskog L, Forsum U. *In situ* identification of T lymphocyte subsets and HLA-DR expressing cells in the human skin tuberculin reaction. Clin Exp Immunol 1982; **49**:325–30.

3 Platt J, Grant BW, Eddy AA, Michael AF. Immune cell population in cutaneous delayed-type hypersensitivity. J Exp Med 1983; **158**:1227–42.

4 Konttinen YT, Bergroth V, Visa-Tolvanen K, Teitamo S, Forstrom L. Cellular infiltrate *in situ* and response kinetics of human intradermal and epicutaneous tuberculin reactions. Clin Immunol Immunopathol 1983; **28**:441–9.

5 Gibbs JH, Ferguson J, Brown RA *et al.* Histometric study of the localisation of lymphocyte subsets and accessory cells in human Mantoux reactions. J Clin Pathol 1984; **37**:1227–34.

6 Fullmer MA, Shen J-Y, Modlin RL, Rea TH. Immunohistological evidence of lymphokine production and lymphocyte activation antigens in tuberculin reactions. Clin Exp Immunol 1987; **67**:383–90.

7 Tsicopoulos A, Hamid Q, Varney V *et al.* Preferential messenger RNA expression of Th1-type cells ($IFN\text{-}\gamma^+$, $IL\text{-}2^+$) in classical delayed-type (tuberculin) hypersensitivity reactions in human skin. J Immunol 1992; **148**:2058–61.

8 Chu CQ, Field M, Feldmann M, Maini RN. Localization of tumour necrosis factor α in synovial tissues and at the cartilage/pannus junction in patients with rheumatoid arthritis. Arthritis Rheum 1991; **49**:1125–32.

9 Huninghake GW, Glazier AJ, Monick MM, Dinarello CA. Interleukin 1 is a chemotactic factor for human T-lymphocytes. Am Rev Respir Dis 1987; **135**:66–71.

10 Ming WJ, Bersani L, Montevani A. Tumour necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. J Immunol 1987; **138**:1469–74.

11 Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K. Production of interleukin-8 by human dermal fibroblasts and keratinocytes

in response to interleukin-1 or tumour necrosis factor. Immunology 1989; **68**:31–36.

12 Larsen CG, Anderson AO, Oppenheim JJ, Matsushima K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. Science 1989; **243**:1464–6.

13 Schall TJ, Bacon K, Toy KJ, Goeddel DV. Selective attraction of monocytes and T lymphocytes of the memory phenotype by cytokine RANTES. Nature 1990; **347**:669–71.

14 Smith KA. T-cell growth factor. Immunol Rev 1980; **51**:337–57.

15 Kasahara T, Hooks JJ, Dougherty SF, Oppenheim JJ. Interleukin 2-mediated immune interferon (IFN-gamma) production by human T cells and T cell subsets. J Immunol 1983; **130**:1784–801.

16 Nathan CF, Murry HW, Wiebe ME, Rubin BY. Identification of interferon as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. J Exp Med 1983; **158**:670–89.

17 Dinarello CA. Interleukin-1 and its biologically related cytokines. Adv Immunol 1989; **44**:153–205.

18 Van Snick J. Interleukin-6: an overview. Annu Rev Immunol 1990; **8**:253–78.

19 Erb P, Feldmann M. Role of macrophages in the *in vitro* induction of T help cells. J Exp Med 1975; **142**:460–72.

20 Springer TA. Adhesion receptors of the immune system. Nature 1990; **346**:425–34.

21 Yu C-L, Haskard DO, Cavender D, Johnson AR, Ziff M. Human gamma interferon increases the binding of T lymphocytes to endothelial cells. Clin Exp Immunol 1985; **62**:554–60.

22 Issekutz TB, Stoltz JM, van der Meide P. The recruitment of lymphocytes into the skin by T cell lymphokines: the role of γ-interferon. Clin Exp Immunol 1988; **73**:70–75.

23 Issekutz TB, Stoltz JM, van der Meide P. Lymphocyte recruitment in delayed-type hypersensitivity. The role of IFN-γ. J Immunol 1988; **140**:2989–93.

24 Munro JM, Pober JS, Cotran RS. Tumour necrosis factor and interferon-gamma induce distinct patterns of endothelial activation and associated leukocyte accumulation in skin of Papio anubis. Am J Path 1989; **135**:121–33.

25 Barker JN, Allen MH, MacDonald DM. The effect of *in vivo* interferon-gamma on the distribution of LFA-1 and ICAM-1 in normal human skin. J Invest Dermatol 1989; **93**:439–42.

26 Dustin ML, Springer TA. Lymphocyte function-associated antigen-1 (LFA-1) interaction with intercellular adhesion molecule-1 (ICAM-1) is one of at least three mechanisms for lymphocyte adhesion to cultured endothelial cells. J Cell Biol 1988; **107**:321–31.

27 Norris P, Poston RN, Thomas DS, Thornhill M, Hawk J, Haskard DO. The expression of endothelial leukocyte adhesion molecule-1 (ELAM-1), intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) in experimental cutaneous inflammation: a comparison of ultraviolet B erythema and delayed hypersensitivity. J Invest Dermatol 1991; **96**:763–70.

28 Wellicome S, Thornhill M, Pitzalis C *et al.* A monoclonal antibody that detects a novel antigen on endothelial cells that is induced by tumour necrosis factor, interleukin-1 and lipopolysaccharide. J Immunol 1990; **144**:2558–65.

29 Cher DJ, Mosmann TR. Two types of murine helper T cell clone. II. Delayed-type hypersensitivity is mediated ty TH1 clones. J Immunol 1987; **138**:3688–94.

30 Shimada S, Katz SI. The skin as an immunologic organ. Arch Pathol Lab Med 1988; **112**:231–4.

31 Larsen CG. Leukocyte activating and chemotactic cytokines in cell-mediated immune reactions of the human skin. Acta Dermatovenereologica 1991; **Suppl. 160**:1–48.

32 Demidem A, Taylor IR, Grammer SF, Streilein JW. T-lymphocyte-activating properties of epidermal antigen-presenting cells from normal and psoriatic skin: evidence that psoriatic epidermal antigen-presenting cells resemble cultured normal Langerhans cells. J Invest Dermatol 1991; **97**:454–60.

33 Sauder DN, Dinarello CA, Morhenn VB. Langerhans cell production of interleukin-1. J Invest Dermatol 1984; **82**:605–7.

34 Larrick JW, Morhenn V, Chiang YL, Shi T. Activated Langerhans cells release tumour necrosis factor. J Leukoc Biol 1989; **45**:429–33.

35 Gothelf Y, Dinarello CA, Yamin M, Sharon N, Milner Y, Gazit E. IL-1 production by T6 (CD1a) positive cord blood mononuclear cells (Langerhan's cell precursors?). Lymphokine Res 1989; **8**:373–82.

36 Brennan FM, Field M, Chu CQ, Feldmann M, Maini RN. Cytokine expression in rheumatoid arthritis. Br J Rheumatol 1991; **30 (Suppl. 1)**:76–80.

37 Cattoretti G, Berti E, Parravicini C *et al.* Expression of CD1 molecules on dendritic cells: ontogeny, epitope analysis of normal and malignant cells, and tissue distribution. In: Knapp W, Dhorken B, Gilks WR *et al.*, eds. Leucocyte typing IV. Oxford: Oxford University Press, 1989:262–4.

38 Chu CQ. Immunohistochemical study of cytokines in rheumatoid arthritis. PhD Thesis. London, University of London, 1991:67–101.

39 Field M, Chu C, Feldmann M, Maini RN. Interleukin-6 localisation in the synovial membrane in rheumatoid arthritis. Rheumatol Int 1991; **11**:45–50.

40 Zheng RQH, Abney ER, Chu CQ *et al.* Detection of *in vivo* production of tumour necrosis factor-alpha by human thyroid epithelial cells. Immunology 1992; **75**:456–62.