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In vivo lymphokine production in experimental autoimmune uveoretinitis

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SUMMARY

Experimental autoimmune uveoretinitis (EAU) is a well-characterized model of immune-mediated intraocular inflammation. The intraocular infiltrate in EAU consists predominantly of T lymphocytes. The *in vivo* production of interleukin-2 (IL-2), lymphotoxin and IL-4 by these T cells was investigated by *in situ* hybridization using cDNA probes to lymphokine mRNA. Localization of lymphokine mRNA was found simultaneous with disease onset in areas of T-cell infiltration. Positive signal was seen over cells in the uveal tract, retina and extraocular region. Less than 10% of the population of T cells defined immunohistochemically had positive localization of mRNA for these lymphokines. The number of positive cells was similar for each of the three probes and increased as the disease progressed. The findings suggest that these lymphokines are produced *in vivo* in immune-mediated intraocular inflammation and may play a role in the immunopathology seen in these conditions.

INTRODUCTION

Experimental autoimmune uveoretinitis (EAU)[1] is a model of human ocular inflammatory disease, a range of conditions which can result in significant visual loss despite treatment. The aetiology of human intraocular inflammation is unknown but autoimmune mechanisms involving ocular antigens have been implicated in its pathogenesis. EAU can be induced in various animal species by systemic immunization with a retinal antigen in adjuvant. In the Lewis rat model of EAU, immunization is followed 10–12 days later by a mixed infiltrate of polymorphonuclear leucocytes and lymphocytes to the anterior and posterior segments of the eye, with CD4+ T lymphocytes predominating in the destructive retinal lesions.[2] It has been demonstrated that activated, retinal antigen-specific CD4+ T cells are capable of adoptively transferring EAU to non-immunized animals.[3] CD4+ T lymphocytes are therefore implicated in both the initiation of the disease process and the destructive retinal pathology.

Murine CD4+ T cells have been divided into two types based on their pattern of lymphokine secretion[4] and this has been correlated with their function. Th1 cells secrete interleukin-2 (IL-2), interferon-γ (IFN-γ) and lymphotoxin and are thought to function as effector cells, whereas Th2 cells produce IL-4, IL-5 and IL-6 and are considered to be helper cells, for example providing specific B-cell help. Both cell types produce IL-3, granulocyte–macrophage colony-stimulating factor (GM-CSF) and tumour necrosis factor-α (TNF-α). Rat CD4+ T cells do not appear to follow this stable pattern of lymphokine secretion and have been subtyped according to their binding of the OX22 monoclonal antibody. Naive CD4+ T cells (OX22 high) have a high binding affinity for this antibody and produce IL-2 and IFN-γ, following antigen contact these cells become OX22 low and produce IL-4.[5–7]

Rat uveitogenic T-cell lines have been shown to produce IL-2, IL-4 and IFN-γ when activated *in vitro*.[8] We have previously demonstrated that there is *in vivo* expression of IFN-γ mRNA and the presence of IFN-γ protein in areas of T-lymphocyte infiltrate in the destructive tissue pathology throughout the time-course of EAU.[9] In this study we have extended these investigations to the lymphokines IL-2, lymphotoxin and IL-4 with the aim of further characterizing the *in vivo* pattern of lymphokine production in the destructive lesions seen in EAU. We have identified infiltrating cells immunohistochemically and have used cDNA probes to detect lymphokine mRNA by *in situ* hybridization.

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MATERIALS AND METHODS

Animals

Female Lewis rats [bred under specific pathogen-free (SPF) conditions; St Thomas' Hospital Medical School, London, U.K.], 100–150 g weight, 6–8 weeks old were used for experiments and as controls.

Induction of uveitis

Rats were immunized in a hind footpad with 50 μg of purified bovine S-antigen[10] in a 1:1 emulsion in complete Freund's adjuvant (CFA) (Sigma, Poole, U.K.) supplemented with

Mycobacterium tuberculosis organisms (Sigma) to a final concentration of 2·5 mg/ml. Animals were also given 5×10^9 heat-inactivated *Bordetella pertussis* organisms (Wellcome Laboratories, Beckenham, U.K.) in 150 μl phosphate-buffered saline (PBS) intraperitoneally. A total of 24 immunized and four control (non-immunized) eyes from individual animals was studied. Two animals were studied on days 10 and 11 post-immunization, and four animals were studied on days 12, 13, 14, 17 and 21 post-immunization. Eyes were rapidly removed, embedded in OCT (Shandon, Runcorn, U.K.) and snap frozen in acetone and dry ice. Specimens were stored at −70°.

Probe preparation

Three cDNA probes were used for the *in situ* hybridization. The rat IL-2 probe was a full-length 740 base pair (bp) fragment corresponding to rat IL-2 mRNA[11] and the rat IL-4 probe consisted of the first 406 bp of the specific rat IL-4 gene structure.[7] Both these were probes kindly supplied by Dr A. McKnight (MRC Cellular Immunology Unit, Oxford, U.K.) The lymphotoxin probe was a 1·42 kbp coding sequence derived from the murine lymphotoxin gene[12] (kindly given by Dr N. Ruddle, Yale University Medical School, New Haven, CT). The probe-containing plasmids were amplified in HB 101 *Escherichia coli* and the cDNA probes were extracted, purified and labelled with ^{35}S dCTP alpha (Amersham International, Amersham, U.K.) using the random primer technique (Boeringer Mannheim, Lewes, U.K.) and adjusted to 2×10^5 c.p.m./ml in hybridization buffer [600 mM sodium chloride, 50 mM sodium phosphate pH 7·0, 5 mM EDTA, 0·02% Ficoll, 0·02% bovine serum albumin (BSA), 0·02% polyvinylpyrolidene, 0·1% salmon testis DNA and 50% deionized formamide (all chemicals supplied by Sigma)].

In situ *hybridization*

Whole eye sections of 12 μm thickness were cut on a cryostat and mounted on specially prepared gelatin (300 bloom swine, Sigma) coated slides. Sections were fixed for 5 min in 4% glutaraldehyde in 0·1 M Sorensens phosphate buffer pH 7·2 with 20% ethylene glycol, rinsed twice in hybridization buffer and soaked in hybridization buffer for 1 hr, rinsed in ethanol and dried. The ^{35}S-labelled probe in hybridization buffer was heated to 90° for 10 min, cooled and 100 μl applied to each slide under a parafilm (Sigma) coverslip. Sections were left to hybridize in a humidified chamber at room temperature for 72 hr. Post-hybridization, slides were immersed in 2 × SSC until the coverslips dislodged, rinsed in 2 × SSC and washed at 40° for 30 min in 1 × SSC. Slides were then rinsed briefly in distilled water, in 70% ethanol for 5 min, in 95% ethanol for 5 min and allowed to dry.

Control sections from each eye were fixed for 5 min in glutaraldehyde buffer, washed once in 2 × SSC with 5% Tween and three times in 2 × SSC. One hundred microlitres of RNAase A (Boehringer Mannheim) 1 mg/ml in 2 × SSC was applied to each slide under a parafilm coverslip and incubated at 37° for 1 hr, slides were then washed in 2 × SSC and simultaneous hybridization with each probe carried out as above.

To determine the specificity of the lymphokine probe hybridization, each section was also hybridized with a ^{35}S-labelled probe to the P53 oncogene which was considered to be irrelevant to the EAU disease process.

Autoradiography

The slides were dipped in K5 (Ilford, Mobberley, U.K.) photographic emulsion diluted 1:1 in 0·5% glycerol and left to expose at 4° for 21–24 days over silica gel. Slides were then developed for 3·5 min in D19 (Kodak, Hemel Hempstead, U.K.) developer, fixed in Unifix (Kodak), washed in distilled water and counterstained with haematoxylin.

To compare the number of cells producing each lymphokine the mean number of positive cells at each stage of the disease process was estimated for each probe.

Monoclonal antibodies

For immunohistochemistry primary monoclonal antibodies to pan rat T cells (OX19; Serotex, Oxford, U.K.), IL-2 receptor (OX39, Serotec) and (MHC) class II expression (OX6; Serotec) were utilized.

Immunohistochemistry

Sections of 6 μm thickness were cut from each eye and mounted on 3-amino propyl triethoxysilane (Sigma) coated slides. Slides were fixed in acetone for 7 min and endogenous peroxide activity blocked with 3% hydrogen peroxide in 50% methanol. Sections were stained by a standard avidin–biotin complex method (Vector, Peterborough, U.K.) using a rat immunoglobulin adsorbed biotinylated secondary antibody (Vector) and amino ethyl carbazole to provide a red final reaction product.

RESULTS

Induction of disease

Animals developed histological evidence of disease on day 12 post-induction. The histological changes were characteristic EAU in rats immunized with S antigen[1] and it is well described that this does not occur in animals immunized with CFA alone.[13–15] There was a marked mixed leucocyte infiltrate of the anterior and posterior segments of the eyes with patches of retinal oedema progressing to a total retinal destruction in which lymphocytes were the predominant infiltrating cell.

Localization of IL-2 mRNA

Positive signal for IL-2 mRNA, observed as collections of dark grains in the photographic emulsion, was found over cells from day 12 post-immunization onwards. In the early phase of the disease there was an accumulation of positive cells around the areas of retinal oedema and destruction (Fig. 1). These positive cells were localized to the inner retina despite the focal necrosis of the photoreceptors. At the early disease stage there were also minimal numbers of positive cells in the choroid and vitreous. In the eyes with established and advanced disease there were numerous positive cells in the retina and vitreous, and markedly increased numbers of positive cells scattered throughout the ciliary body and choroid. No positive signal was seen on cells in the anterior chamber. Numerous positive cells were seen in the extraocular inflammatory infiltrates in both anterior and posterior extraocular regions at all stages of the disease process.

Localization of lymphotoxin mRNA

Positive hybridization signal from the lymphotoxin probe was generally less strong than the other probes utilized. In the early

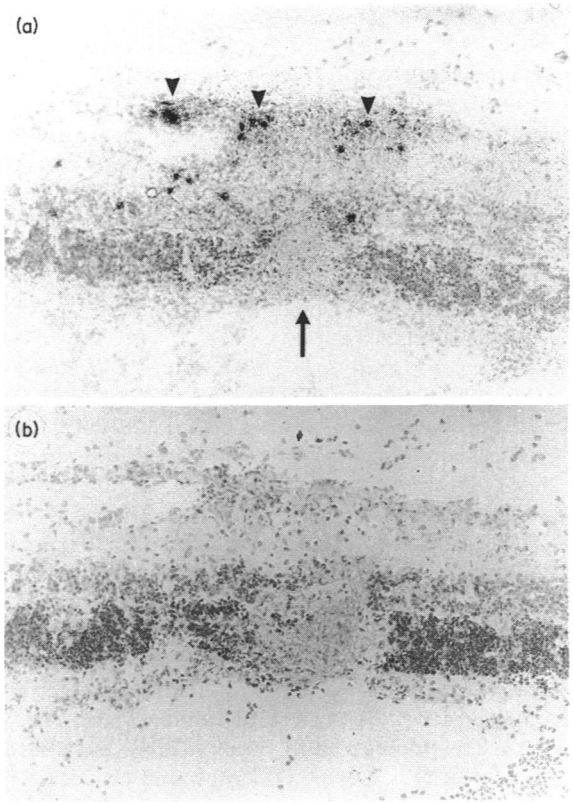

Figure 1. (a) Detached retina in early (day 12 post-immunization) EAU. Cells in inner retina have positive hybridization signal to IL-2 probe (arrowheads to examples) around a focus of outer retinal oedema and destruction (arrow). Haematoxylin counterstain × 120. (b) Adjacent section to (a) pre-treated with RNAase. No localization of IL-2 mRNA. Haematoxylin counterstain × 120.

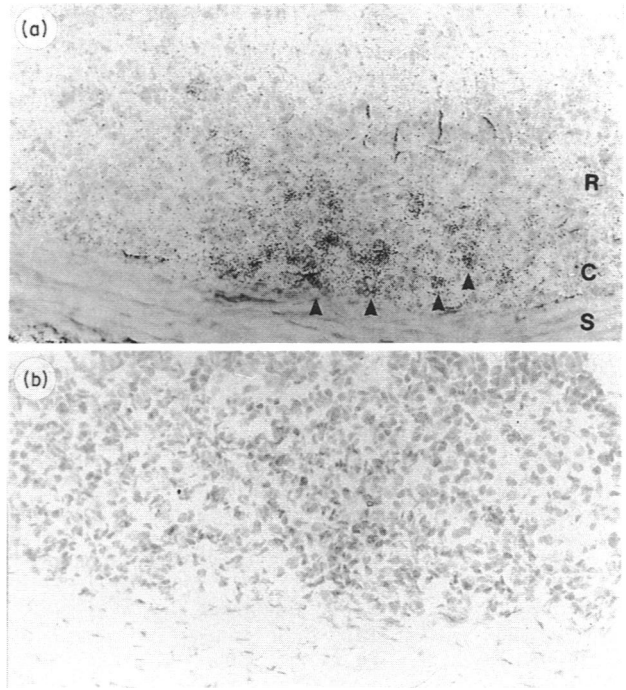

Figure 2. (a) Foci of localization of lymphotoxin mRNA (arrowheads to examples) in outer retina (R) and choroid (C) day 14 post-immunization. S = sclera. Haematoxylin counterstain × 120. (b) Adjacent section to (a) pretreated with RNAase. No localizing signal. Haematoxylin counterstain × 120.

phase of the disease positive cells were most numerous in the extraocular inflammatory cell infiltrates but a few scattered positive cells were also found in the choroid and very occasional positive cells in the retina at this stage (Fig. 2). Numbers of positive cells increased as the disease progressed and were prominent in the choroidal infiltrate in the later phase of the disease. In general very few lymphotoxin positive cells were found in the retina. No positive cells were found in the anterior chamber.

Localization of IL-4 mRNA

Hybridizations with the IL-4 probe revealed positive cells from day 12 post-immunization onwards. These were seen in the inflamed retina (mainly the inner retina, related to patches of retinal necrosis and destruction), choroid and extraocular infiltrates in the early phase of the disease. In the established and late stages of the disease (Fig. 3) there was a marked increase in the numbers of positive cells found in the ciliary body and choroid. Small numbers of cells in the retina and subretinal fluid were positive in the later stages of the disease process. Again, no positive signal was seen on any of the numerous inflammatory cells in the anterior chamber.

Overall results of *in situ* hybridization

Positive cells for each probe were found simultaneous with the onset of disease on day 12 post-immunization, increased in numbers during the established phase of the disease and remained constant during the recovery phase. A similar number of cells were positive for each of the three probes at all stages of the disease. On the frozen sections examined immunohistochemically it was not possible to make accurate cell counts of the T-cell numbers because of the difficulty in defining individual cells with positive immunohistochemical stain in areas of marked accumulation of infiltrating mononuclear cells. It was estimated that 5–10% of the T-cell population had positive localizing signal for each probe.

No autoradiographic signal suggestive of localization of lymphokine mRNA was found using any of the probes in any of the four non-immunized animals studied.

Analysis of sections pretreated with RNAase at a concentration of 1 mg/ml for 1 hr showed that this abolished all localizing signal on the sections of all the positive eyes for each probe (Figs 2b, 3b). Sections hybridized with the P53 probe revealed a generalized background signal over areas of inflamed tissue without any focal cellular localization (not shown).

Immunohistochemistry

OX19 (pan T cell) positive cells were found in inflammatory infiltrates throughout the extraocular tissues, anterior chamber, ciliary body, choroid and retina, including the areas of lymphokine mRNA localization (not shown). Cells in these regions also

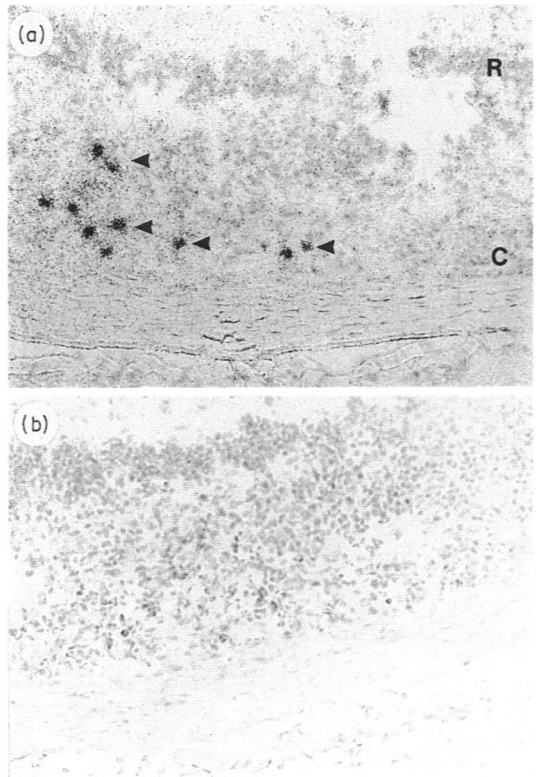

Figure 3. (a) Localizing foci of IL-4 mRNA localization in retina and choroid (C) (arrowheads) in advanced (day 21 post-immunization) EAU. Haematoxylin counterstain × 180. (b) Adjacent section to (a) pretreated with RNAase. No localizing signal with IL-4 probe. Haematoxylin counterstain × 180.

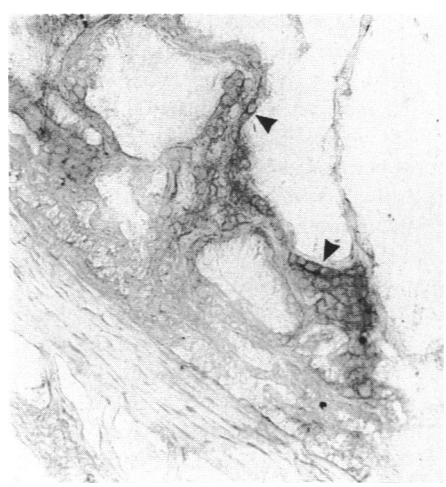

Figure 4. IL-2 receptor (OX39) positive T lymphocytes (arrowheads) infiltrating the ciliary body in advanced (day 17) disease. Haematoxylin counterstain × 150.

had positive staining with the OX39 antibody for IL-2 receptor expression (Fig. 4). MHC class II expression was found on cells from day 10 post-induction onwards, this increased markedly as the disease progressed and was seen both on organ-resident and infiltrating cells.

DISCUSSION

The presence of mRNA for the lymphokines IL-2, lymphotoxin and IL-4 was demonstrated in the areas of T-lymphocyte infiltrate in actively inflamed eyes in EAU. We have previously demonstrated that IFN-γ mRNA and protein are present in a similar distribution.[9] At present no specific monoclonal antibodies are available for the immunohistochemical detection of rat IL-2, lymphotoxin and IL-4 protein but the other investigations have shown that levels of IL-2, IL-2 receptor and IFN-γ protein[16] and bioactive IL-2 and IL-4[17] reflects the level of their respective cellular mRNA.

The presence of these four cytokines has implications with regard to the subsets of CD4$^+$ T cells (the predominant infiltrating cell type in EAU) present in the rat model used. If rat CD4$^+$ T cells conform to the pattern of subsets found in murine CD4$^+$ T-cell clones, that is the Th1 and Th2 types,[4] then it follows that both subsets are present in the T-cell infiltrates in this model. Alternatively it is possible that the T cells present are of the Th0 subset which are thought to be a product of an acute or short-term stimulation and produce IFN-γ, IL-2 and IL-4.[18] There is evidence, however, that rat CD4$^+$ T cells do not fit in to the stable pattern of lymphokine secretion seen in murine CD4$^+$ T-cell clones. Rat CD4$^+$ T cells have been subtyped according to their binding of the OX22 monoclonal antibody, naive CD4$^+$ T cells (OX22 high) produce IL-2 and IFN-γ and following antigen contact cells become OX22 low and produce IL-4.[5-7] According to this scheme both naive and memory CD4$^+$ T cells would be present in the infiltrates found in EAU. This would seem logical since any section of an actively inflamed eye would be likely to contain a mixture of T cells involved in the dynamic process of trafficking to the eye followed by antigen contact and subsequent effector or helper function. It is also possible that additional subgroups of rat CD4$^+$ T cells exist and the position may be considerably more complex. Analysis of the cytokine secretion by individual T cells from the inflammatory infiltrates would be necessary to investigate further the exact nature of the T cells present. Additionally non-ocular antigen-specific T cells may be involved in the destructive process following chemoattraction produced by activated ocular antigen-specific T cells.

The IL-2 and IL-4 producing cells were notably distributed in relation to foci of retinal oedema and destruction suggesting that a stimulus to produce these lymphokines is present in this area and their subsequent release may contribute to the focal destructive lesions seen in the early stages of EAU. Antigen contact is one potential stimulus; however, it is notable that these cells were distributed almost entirely within the inner retina in contrast to the outer retinal distribution of S antigen. The stimulus for production of these lymphokines may therefore be another antigen or inflammatory mediator.

IL-2 is a lymphokine which is produced by helper T cells following activation by antigenic or mitogenic stimulation; it is required for the subsequent proliferation of T lymphocytes and therefore plays a key role in the initiation of an immune response.[19] IL-2 mRNA has been demonstrated at the sites of tissue pathology in autoimmune disease such as rheumatoid arthritis.[20] IL-2 protein has been demonstrated in human eyes with posterior uveitis[21] and an increase in circulating IL-2 receptor positive[22] T lymphocytes has been demonstrated in patients with active uveitis. The demonstration of IL-2 mRNA and IL-2 receptor positive lymphocytes in EAU is further

evidence that this mediator is central to the immunopathology in intraocular inflammatory disease.

Lymphotoxin is produced by both $CD4^+$ and $CD8^+$ T lymphocytes and is thought to play a key role in T-lymphocyte cytotoxicity.[23] Secretion of IFN-γ along with lymphotoxin appears to promote $CD4^+$, class II-restricted T-cell cytotoxicity.[24] IL-2 has also been demonstrated to produce differentiation of cytotoxic T lymphocytes.[25,26] The *in vivo* expression of mRNA specific for these lymphokines at the site of tissue pathology in EAU is evidence that the tissue destruction may be produced by effector T lymphocytes.

T-cell cytotoxicity can be mediated by several mechanisms[24,27-29] and the relatively low numbers of cells in the retinal pathology producing lymphotoxin would suggest that cytotoxic mechanisms other than those involving lymphotoxin may be involved.[30] The widespread cellular expression of MHC class II antigens in the eye in EAU would provide suitable target cells for cytotoxic $CD4^+$ T cells which may subsequently produce more extensive damage through 'innocent bystander' killing.[24]

IL-4 produces B-lymphocyte activation, MHC class II expression and immunoglobulin (IgG1 and IgE) production. It is also involved in T-lymphocyte activation and growth.[31] Although few B cells are present in the intraocular pathology in EAU, *in vivo* IL-4 production would facilitate antigen presentation by those B cells present to the infiltrating antigen-specific T lymphocytes.

IL-4 has been shown to increase the cytotoxic capacity and MHC class II expression of macrophages.[31] IFN-γ also has the potential to activate macrophages[32] and the *in vivo* production of these two lymphokines could therefore promote an effector cell function in the numerous macrophages present at the sites of tissue pathology in EAU.

It has been demonstrated that mRNA for multiple cytokines is present in affected joints in rheumatoid arthritis,[33] a disease thought to have an autoimmune aetiology. Our results indicate that this may also be the case in intraocular inflammatory disease and the relative contribution of each individual cytokine to the tissue pathology will require further study.

Targeting the lymphokines involved in the pathogensis of immune-mediated pathology may limit the subsequent tissue destruction. Several such approaches are currently under investigation, for example the use of monoclonal antibodies to a cytokine or its receptor,[34] the administration of soluble cytokine receptors[35] to interfere with the cytokine–receptor interaction or the use of specific cytokine antagonists.[36] Further investigation of the cytokine mechanisms involved in EAU will be useful in the development of more specific therapies for human intraocular inflammatory disease.

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REFERENCES

1. Gery I., Mochizuki M. & Nussenblatt R.B. (1986) Retinal specific antigens and immunopathogenic processes they provoke. In: *Progress in Retinal Research* (eds N. N. Osborne and G. J. Chader), edn 5, p. 75. Pergamon Press, Oxford.
2. Chan C.C., Mochizuki M., Nussenblatt R.B., Palestine A.G., McAllister C., Gery I. & Benezra D. (1985) T-lymphocyte subsets in experimental autoimmune uveitis. *Clin. Immunol. Immunopathol.* **35,** 103.
3. Caspi R.R., Roberge F.G., McAllister C.G., El-Saied M., Kuwabara T., Gery I., Hanna E. & Nussenblatt R.B. (1986) T cell lines mediating experimental autoimmune uveoretinitis (EAU) in the rat. *J. Immunol.* **136,** 928.
4. Mosmann T.R., Cherwinski H., Bond M.W., Giedlin M.A. & Coffman R.L. (1986) Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J. Immunol.* **136,** 2348.
5. Powrie F. & Mason D. (1988) Phenotypic and functional heterogeneity of $CD4^+$ T cells. *Immunol. Today*, **9,** 274.
6. Powrie F. & Mason D. (1989) The MRC $OX\text{-}22^-$ $CD4^+$ T cells that help B cells in secondary immune responses derive from naive precursors with the MRC $OX\text{-}22^+$ $CD4^+$ phenotype. *J. exp. Med.* **169,** 653.
7. McKnight A.J., Barclay A.N. & Mason D.W. (1991) Molecular cloning of rat interleukin 4 cDNA and analysis of the cytokine repertoire of subsets of $CD4^+$ T cells. *Eur. J. Immunol.* **21,** 1187.
8. Lightman S.L., Caspi R. & Nussenblatt R.N. (1989) Lymphokine secretion by a $CD4^+$ uveitogenic T cell line. *Invest. Ophthalmol. vis. Sci.* **30,** 278.
9. Charteris D.G. & Lightman S.L. (1992) Interferon-gamma (IFN-γ) production *in vivo* in experimental autoimmune uveoretinitis. *Immunology*, **75,** 463.
10. Dorey C., Cozette J. & Faure J.P. (1982) A simple and rapid method for isolation of retinal S antigen. *Ophthalmic Res.* **14,** 249.
11. McKnight A.J., Mason D.W. & Barclay A.N. (1989) Sequence of rat interleukin 2 and anomalous binding of a mouse interleukin 2 cDNA probe to rat MHC class II-associated invariant chain mRNA. *Immunogenetics*, **30,** 145.
12. Li C.B., Gray P.W., Lin P.F., McGrath K.M., Ruddle F.H. & Ruddle N.H. (1987) Cloning and expression of murine lymphotoxin cDNA. *J. Immunol.* **138,** 4496.
13. Waksman B. & Bullington S. (1960) Studies of arthritis and other lesions induced in rats by injection of mycobacterial adjuvant: lesions of the eye. *Arch. Ophthalmol.* **64,** 751.
14. Petty R.E., Johnston W., McCormick A.Q., Hunt D.W.C., Rootman J. & Rollins D.F. (1989) Uveitis and arthritis induced by adjuvant: clinical, immunologic and histologic characteristics. *J. Rheumatol.* **16,** 499.
15. Lightman S. & Greenwood J. (1992) Effect of lymphocytic infiltration on the blood–retinal barrier in experimental autoimmune uveoretinitis. *Clin. exp. Immunol.* **88,** 473.
16. Grabstein K., Dower S., Gillis S., Urdal D. & Larsen A. (1986) Expression of interleukin 2, interferon-γ, and the IL2 receptor by human peripheral blood lymphocytes. *J. Imunol.* **136,** 4503.
17. Carding S.R., West J., Woods A. & Bottomly K. (1989) Differential activation of cytokine genes in normal CD4-bearing T cells is stimulus dependent. *Eur. J. Immunol.* **19,** 231.
18. Mosmann T.R. & Moore K.W. (1991) The role of IL-10 in crossregulation of T_H1 and T_H2 responses. *Immunol. Today*, **7,** 49.
19. Smith K.A. (1988) Interleukin-2: inception, impact, and implications. *Science*, **240,** 1169.
20. Warren C.J., Howell W.H., Bhambhani M., Cawley I.D. & Smith J.L. (1991) An investigation of T-cell subset phenotype and function in the rheumatoid synovium using *in situ* hybridization for IL-2 mRNA. *Immunology*, **72,** 250.
21. Hooks J.J., Chan C.C. & Detrick B. (1986) Identification of the lymphokines interferon gamma and interleukin 2 in inflammatory eye diseases. *Invest. Ophthalmol. vis. Sci.* **29,** 1444.
22. Deschenes J., Char D.H. & Kaleta S. (1988) Activated T lymphocytes in uveitis. *Br. J. Ophthalmol.* **72,** 83.
23. Paul N.L. & Ruddle N.H. (1988) Lymphotoxin. *Ann. Rev. Immunol.* **6,** 407.
24. Tite J.P., Powell M.B. & Ruddle N.H. (1985) Protein-antigen specific Ia-restricted cytolytic T cells: analysis of frequency, target cell susceptibility, and mechanism of cytolysis. *J. Immunol.* **135,** 25.
25. Simon M.M., Landolfo S., Diamantstein T. & Hochgesch-

WENDER U. (1986) Antigen- and lectin-sensitized murine cytolytic T lymphocyte-precursors require both interleukin 2 and endogenously produced immune (γ) interferon for their growth and differentiation into effector cells. *Curr. Top. Microbiol. Immunol* **126,** 173.

26. HAWORTH C. & FELDMANN M. (1989) Lymphokines and disease. In: *T cells* (eds M. Feldmann, J. Lamb and M. J. Owen) p. 255. Wiley & Sons, New York.
27. TITE J.P. (1990) Differential requirement for protein synthesis in cytolysis mediated by class I and class II MHC-restricted cytotoxic T cells. *Immunology*, **70,** 440.
28. TITE J.P. (1990) Evidence of a role for TNF-α in cytolysis by $CD4^+$, class II MHC-restricted cytotoxic T cells. *Immunology*, **71,** 208.
29. LANCKI D.W., HSIEH C.S. & FITCH F.W. (1991) Mechanisms of lysis by cytotoxic T lymphocyte clones. *J. Immunol.* **146,** 3242.
30. JONGENEEL C.V., NEDOSPASOV S.A., PLAETINCK G., NAQUET P. & CEROTTINI J.C. (1988) Expression of the tumor necrosis factor locus is not necessary for the cytolytic activity of T lymphocytes. *J. Immunol.* **140,** 1916.
31. PAUL W.E. & OHARA J. (1987) B-cell stimulatory factor-1/interleukin-4. *Ann. Rev. Immunol.* **5,** 429.
32. NATHAN C.F., MURRAY H.W., WIEBE M.E. & RUBIN B.Y. (1983) Identification of interferon-gamma as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. exp. Med.* **158,** 670.
33. FELDMANN M., BRENNAN F.M., CHANTRY D., HAWORTH C., TURNER M., ABNEY E. *et al.* (1990) Cytokine production in the rheumatoid joint: implications for treatment. *Ann. Rheum. Dis.* **49,** 480.
34. KELLEY V.E., GAULTON G.E., HATTORI M., IKEGAMI H., EISENBARTH G. & STROM T.B. (1988) Anti-interleukin 2 receptor antibody suppresses murine diabetic insulitis and lupus nephritis. *J. Immunol.* **140,** 59.
35. FANSLOW W.C., SIMS J.E., SASSENFELD H., MORRISSEY P.J., GILLIS S., DOWER S.K. & WIDMER M.B. (1991) Regulation of alloreactivity *in vivo* by a soluble form of the interleukin-1 receptor. *Science*, **248,** 739.
36. OHLSSON K., BJORK P., BERGENFELDT M., HAGEMAN R. & THOMPSON R.C. (1990) Interleukin-1 receptor antagonist reduces mortality from endotoxin shock. *Nature*, **348,** 550.