

Melanoma specific Th1 cytotoxic T lymphocyte lines in Vogt-Koyanagi-Harada disease

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Abstract

Aims/background—To determine the functional properties and cytokine production profiles of melanoma specific cytotoxic T lymphocytes (CTLs) induced from peripheral blood leucocytes of two patients with Vogt-Koyanagi-Harada disease (VKH).

Methods—Melanoma specific CTL lines were established by long term coculture with a human melanoma cell line (P-36). Cytotoxic activity against P-36 was measured by ^{51}Cr release. The involvement of human leucocyte antigen (HLA) class I or class II molecules in the cytotoxicity of the CTL lines against P-36 was analysed using anti-HLA class I or anti-HLA class II monoclonal antibody (MAb). Surface molecules of CTL lines were analysed by flow cytometry using MAbs specific for CD4, CD8, CD16, CD25, CD56, HLA-DR, T cell antigen receptor (TCR) $\alpha\beta$ and TCR $\gamma\delta$. Cytokine production and soluble interleukin 2 receptor (sIL-2R) secretion were determined by enzyme linked immunosorbent assays. mRNAs of cytokines were analysed using reverse transcription polymerase chain reaction (RT-PCR).

Results—CTLs showed strong cytotoxic activity against P-36. The CTL activity of the cell lines against P-36 was inhibited by the anti-HLA-DR MAb, whereas the MAb specific for monomorphic determinants of HLA-A, B, and C failed to block lytic activity. Flow cytometry identified the following surface molecules: CD4 $^{+}$, CD8 $^{-}$, CD16 $^{-}$, CD25 $^{+}$, CD56 $^{-}$, HLA-DR $^{+}$, TCR $\alpha\beta$ $^{+}$, and TCR $\gamma\delta$. CTLs constitutively produced a high level of IL-6. IL-6 production and sIL-2R secretion of CTLs were enhanced when CTLs were stimulated with P-36. CTLs also produced high levels of interferon γ (IFN- γ) and IL-2, but not IL-4. mRNAs of IL-2 and IFN- γ were detected by RT-PCR in the CTLs.

Conclusions—Melanoma specific HLADR restricted T helper 1 (Th1) CTLs may play a role in the immunopathogenesis of VKH. (*Br J Ophthalmol* 1996;80:1002-1008)

that contain melanocytes. Immunopathological examination of eyes from patients with VKH has shown infiltration of numerous types of leucocytes, especially T lymphocytes,^{1,2} indicating that VKH is an immunogenic inflammation disorder. Melanocyte specific cytotoxic T lymphocytes (CTLs) are believed to be generated in VKH and to play an important role in disease manifestations.³ The frequency of melanocyte specific T cells in the blood was relatively high in the blood from the patients with VKH.³ Many types of T cells are contained in inflammatory tissues, such as the peripheral blood, the aqueous humour, and the uvea. Thus, identification of the subset of antigen specific CTLs involved in VKH is important. Examination of cloned T cells that react to melanocytes would help to identify this subset.

Cytokines are believed to play major roles in immune responses in uveitic eyes,^{1,4} primarily by regulating the diverse functions of lymphocytes and monocytes. The type of antigen specific immune response is determined by the secretion or preferential activation of subsets of CD4 $^{+}$ T cells. T helper 1 (Th1), Th2, and Th0 cells secrete distinctive subsets of cytokines that are associated with different T cell functions.⁵

In the present study, we established cell lines of melanoma specific CTLs using peripheral blood leucocytes (PBLs) obtained from two patients with VKH for the purpose of studying cell functions, surface markers, and patterns of cytokine secretion in these cell lines.

Materials and methods

BLOOD DONORS

Two Japanese patients were admitted to the Department of Ophthalmology of the Shinshu University School of Medicine with blurred vision, central scotoma, metamorphopsia, photophobia, and severe headaches. VKH was diagnosed based on clinical symptoms and the results of a slit-lamp examination, ophthalmoscopy, fluorescein angiography, and laboratory analysis of cerebrospinal fluid. The patients had not been receiving systemic corticosteroids. The human leucocyte antigen (HLA) phenotypes of these patients were as follows: patient 1 (SF male; 42 years) HLA-A24, A31, B7, B54, C1, C7, DR1, DR4, DRw53, DQ5,

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Vogt-Koyanagi-Harada disease (VKH) is a systemic disorder that affects various organs

and DQ7; and patient 2 (TS male; 39 years) HLA-A24, B61, B62, Cw3, DR4, DR2, DRw53, DQ1, and DQ3. The control group consisted of six HLA-DR4 matched healthy volunteers (two men and four women; aged 44.7 (SD 6.3) years). The tenets of the Declaration of Helsinki were followed. Informed consent was obtained after the nature of the procedure was fully explained.

TARGET CELLS

The following cell lines were used in the cytotoxicity assays: a human melanoma cell line (P-36; SK-MEL-28; HLA phenotypes: HLA-A11, A26, B40, DR4) originally established at the Memorial Sloan-Kettering Cancer Center (New York, USA) and a human cervical carcinoma cell line (HeLa-S3; HLA phenotypes analysed with polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method: HLA-DRB1*0102/0102). Both cell lines were maintained in RPMI 1640 medium (Gibco, Grand Island, NY, USA) supplemented with 10% fetal calf serum (FCS; Gibco) and penicillin (100 U/ml) at 37 °C in an atmosphere of 5% carbon dioxide in 100% humidity.

ISOLATION OF PBLs AND ESTABLISHMENT OF CELL LINES

PBLs were isolated as described previously.³ In brief, PBLs were isolated from heparinised venous blood by Ficoll-Conray density centrifugation. They were washed three times with Hanks' medium and then suspended in RPMI 1640 medium supplemented with 10% FCS and penicillin (100 U/ml). PBLs were adjusted to a density of 1.0×10^6 cells/ml of medium supplemented with 5 U/ml of recombinant interleukin 2 (rIL-2) (Shionogi, Osaka, Japan). To propagate cell lines, mitomycin C treated P-36 cells were added to the culture medium to serve as the antigen. Growing CTLs were stimulated with the antigen weekly and fed with fresh medium supplemented with 5 U/ml of rIL-2, as needed, according to the growth rate of the culture.^{6,7} Cultures were maintained under the above mentioned conditions.

MEASUREMENT OF CYTOTOXICITY AGAINST P-36 AND HELA-S3

Details of the assay methods have been described elsewhere.³ In brief, 5×10^6 target cells in 0.5 ml of RPMI 1640 medium supplemented with 10% FCS were incubated with 1.85 MBq ⁵¹Cr (sodium chromate, specific activity 16.48 GBq/mg; NEN Products, Boston, MA, USA) for 45 minutes at 37 °C, with occasional gentle shaking. To measure cytotoxicity in PBLs and cell lines, 5×10^4 labelled target cells in 0.2 ml of the medium were placed in 96 well round bottomed microtitre plates (Nunc, Roskilde, Denmark) at effector/target (E/T) ratios of 10:1 and 50:1 for PBLs and 5:1 and 25:1 for cell lines. Experiments were conducted in triplicate. Culture plates were incubated for 6 hours at 37 °C in 5% car-

bon dioxide and 100% humidity. The plates were then centrifuged at 170 g for 10 minutes. A 0.1 ml volume of the supernatant fluid was extracted from each well, and the amount of radioactivity in those samples was counted with a well-type Packard scintillation counter. Specific ⁵¹Cr release was calculated as follows: $(E - C)/(M - C) \times 100$, where E = experimental, C = spontaneous, and M = maximum ⁵¹Cr release by saponin. Cell viability was determined by the trypan blue dye exclusion method. PBLs obtained from six HLA-DR4 matched healthy controls were analysed in a similar manner.

The supernatants from the culture of the CTLs were added to ⁵¹Cr labelled P-36 to investigate the cytotoxic activity of the soluble factors. The cytotoxic activity of the soluble factors in the supernatants was measured by the method described above.

To test the blocking effects of anti-HLA class I or anti-HLA class II monoclonal antibody (MAb), 50 µl of the appropriately diluted MAb was mixed with ⁵¹Cr labelled P-36 or HeLa-S3 target cells and effector cells in a 6 hour CTL assay. The E/T ratios used were 50:1 for PBLs and 10:1 for cell lines.

FLOW CYTOMETRIC ANALYSIS

Flow cytometry of cell populations was performed in two colours with fluorescein isothiocyanate (FITC) or phycoerythrin (PE) conjugated MAbs against human leucocyte antigens. Preparations were incubated at 4 °C and washed in phosphate buffered saline (PBS) (-) containing 1% bovine serum albumin (BSA) and 0.1% sodium azide. Samples were analysed with a FACScan flow analyser (Becton-Dickinson, Mountain View, CA, USA).

ANTIBODIES

MAbs specific for human T cells and T cell subsets were purchased from Becton-Dickinson (Sunnyvale, CA, USA), Ortho Diagnostic Systems (Raritan, NJ, USA), and T Cell Sciences (Cambridge, MA, USA). We used MAbs directed against the lineage markers CD4 (helper/inducer T lymphocytes), CD8 (cytotoxic/suppressor T lymphocytes), CD16 (natural killer (NK) cells), CD25 (IL-2R; activated T and B lymphocytes), CD56 (NK cells), HLA-DR (activated T lymphocytes, B lymphocytes, monocytes, and macrophages), T cell antigen receptor (TCR) $\alpha\beta$ and TCR $\gamma\delta$.

The B33.1.3 MAb (IgG2a), which is specific for HLA-DR, was kindly provided by Dr P Bice, Jefferson Cancer Institute, PA, USA. The W6/32 MAb (IgG2a), which is reactive against monomorphic determinants of HLA-A, B, and C, was purchased from the American Type Culture Collection (ATCC, Rockville, MD, USA). MAbs were used in the ascites form purified by 50% ammonium sulphate precipitation and by passage through an ABx column (J T Baker Inc, Phillipsburg, NJ, USA).

The TCR V region of CTL cell lines was analysed with the Diversi-T $\alpha\beta$ TCR Screening Panel 1A (T Cell Sciences, Inc, USA).

ENZYME LINKED IMMUNOSORBENT ASSAY

Serum samples were obtained from these patients and healthy controls by separating clotted whole blood by centrifugation at 3000 rpm for 10 minutes. Supernatants of cell lines were collected on the third day of culture with or without antigen stimulation and stored at -80°C until assayed for cytokines and soluble IL-2 receptors (sIL-2R). Commercially available double sandwich ELISA test kits were used to measure IL-2 (Otsuka, Tokyo, Japan), IL-4 (Amersham International, Bucks), IL-6 (Toray-Fuji Bionics, Tokyo, Japan), interferon (IFN)- γ (Toray-Fuji Bionics), and sIL-2R (T Cell Diagnostics, Cambridge, MA, USA). Before IL-2 was assayed, anti-IL-2 MAb coated 96 well microplates were washed three times with Dulbecco's PBS (-) containing 0.05% Tween 20. A 200 μl amount of the samples or diluted standards was placed into each well and allowed to react overnight at 37°C . After wells were washed three times, 100 μl of rabbit anti-human IL-2 polyclonal antibody was added and allowed to react for 2 hours at room temperature. Wells were again washed three times and 100 μl of horseradish peroxidase conjugated goat anti-rabbit IgG antibody was added. After incubation, 100 μl of the substrate solution (1 mg/ml *o*-phenylenediamine) was added. The reaction was terminated by adding 100 μl of 1.0 N H_2SO_4 . The final absorbance was measured at 490 nm using an ELISA autoreader (TOSO, Tokyo, Japan). The lowest detectable concentration of IL-2 was 5 pg/ml. IL-4, IL-6, IFN- γ , and sIL-2R were also measured by double sandwich ELISA test kits. The lowest detectable concentrations of IL-4, IL-6, IFN- γ , and sIL-2R were 3 pg/ml, 5 pg/ml, 15 pg/ml, and 85 U/ml, respectively.

REVERSE TRANSCRIPTION POLYMERASE CHAIN REACTION (RT-PCR) FOR CYTOKINE MRNA

CTL lines were cocultured with P-36 for 24 hours. An amount of 1×10^6 cells were harvested and total RNA was prepared by a single step guanidium isothiocyanate-phenol-chloroform extraction method (TRIzol, Gibco BRL, Gaithersburg, MD, USA). Preparations of cDNAs and PCR for detection of IL-2 mRNA, IFN- γ mRNA, and of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA were performed using a TaKaRa RNA PCR kit with AMV RTase (TaKaRa Shuzo Co, Kyoto, Japan). GAPDH DNA was amplified as a standard to ensure that cDNA concentrations in different reaction mixtures were approximately equal. PCR primers used were as follows: GAPDH sense primer 5'-ACCACAGTCCATGCCATCAC-3' and antisense primer 5'-TCCACCACCTGTTGCTGTGA-3'⁸ (Clontech Laboratories, Inc, Palo Alto, CA, USA); IFN- γ sense primer 5'-ATGAAATATACAAGTTATATCTTGGCTTT-3' and antisense primer 5'-GATGCTCTTCGACCTCGAAACAGCAT-3' (Clontech Laboratories, Inc); IL-2 sense primer 5'-CATTGCACTAAGTCTTGCACTTGTC-3' and antisense primer 5'-CGTTGATATTGCTGATTAAGTCCCTG-3' (Clontech Laboratories, Inc). Reactions were incubated

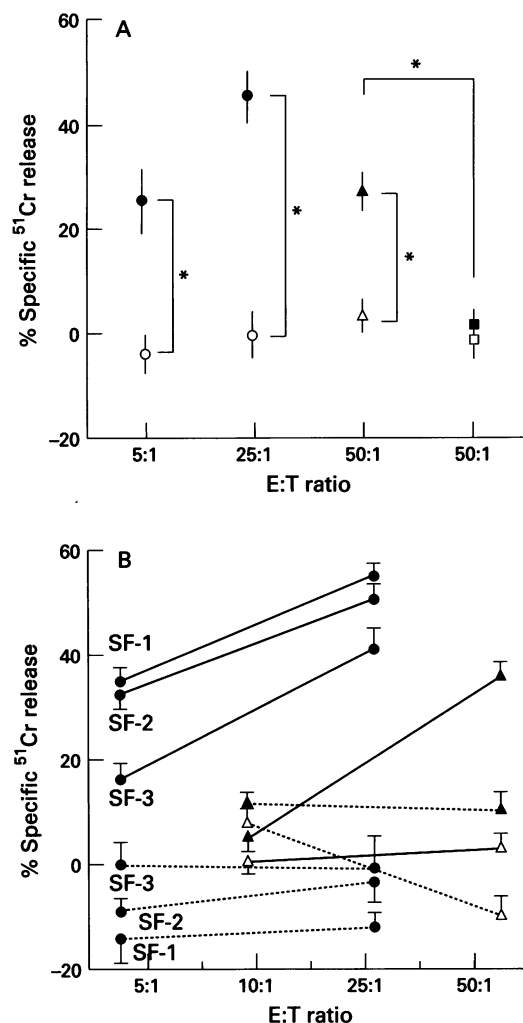


Figure 1 The cytotoxic activity of cytotoxic T lymphocyte (CTL) lines against P-36. (A) The cytotoxic activity of the CTLs (circles) and peripheral blood leucocytes (PBLs) (triangle) obtained from the two patients with VKH and healthy controls (square) against P-36 (closed symbols) and HeLa-S3 (open symbols) was examined by ^{51}Cr release assay. * $p < 0.01$. (B) The cytotoxic activity of the representative SF cell lines (SF-1, -2, and -3) (closed circles) and PBLs obtained from the patient (closed triangles) and of PBLs obtained from healthy controls (open triangles) against P-36 (—) and HeLa-S3 (---) was examined by ^{51}Cr release assay.

in a DNA thermal sequencer (Perkin Elmer Cetus, Norwalk, CT, USA) for 36 cycles. PCR products were run on a 1.2% agarose gel and stained with ethidium bromide.

STATISTICS

Data were shown as mean (SD) in the groups of more than three samples. Results were analysed using the two tailed Student's *t* test, paired or unpaired as appropriate. A *p* value of 0.05 or less was considered significant.

Results

Melanoma specific six IL-2 dependent CTL lines were isolated from the blood of the patients with VKH. These cell lines showed strong cytotoxic activity against P-36 but not against HeLa-S3 (Fig 1A). Cell lysis was efficient even at an E:T ratio of 5:1 and was specific for P-36. The representative data of cytotoxicity against P-36 of CTLs from patient 1 are shown in Figure 1B. P-36 specific cytotoxic activity was not observed in the PBLs from HLA-DR4 matched healthy volunteers.

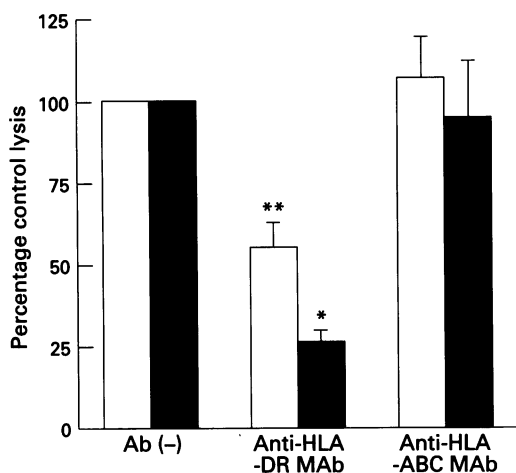


Figure 2 Inhibition of PBLs and SF-1 lysis by anti-HLA class II MAb. P-36 cells were exposed to SF-1 CTLs (solid column) or PBLs (open column) with the anti-HLA-DR MAb (B33.1.3), the anti-HLA-A, B, and C MAb (W6/32), or no Ab (Ab(-)). Data are expressed as percentage control lysis. Significant inhibition of cytotoxic activity of CTL lines (* $p < 0.01$) and PBLs (** $p < 0.05$) by anti-HLA-DR MAb was observed.

The CTL activity of these cell lines and PBLs against P-36 was inhibited by the anti-HLA-DR MAb (B33.1.3) (Fig 2) but not by the MAb (W6/32) specific for monomorphic determinants of HLA-A, B, and C. Thus, antigen recognition by these cell lines was restricted by HLA-DR molecules.

The supernatants from the culture of the cell lines stimulated with P-36 showed no cytotoxicity against P-36, even after 6 hours of in vitro culture (data not shown).

Flow cytometry showed that these cell lines were strongly positive for CD4, CD25, HLA-DR, and TCR $\alpha\beta$ surface molecules but negative for CD8 and TCR $\gamma\delta$ surface molecules (Fig 3). Cell lines were also negative for CD16 and CD56 (data not shown). No positive staining for $\beta V5(a)$, $\beta V5(b)$, $\beta V8(a)$, $\beta V12(a)$, $\beta V6(a)$, $\alpha V2(a)$, or $\alpha\beta V(a)$ TCRs was observed (data not shown).

These cell lines constitutively produced high levels of IL-6 (Table 1). IL-6 production and sIL-2R secretion were enhanced when CTL lines were stimulated with P-36. These cell

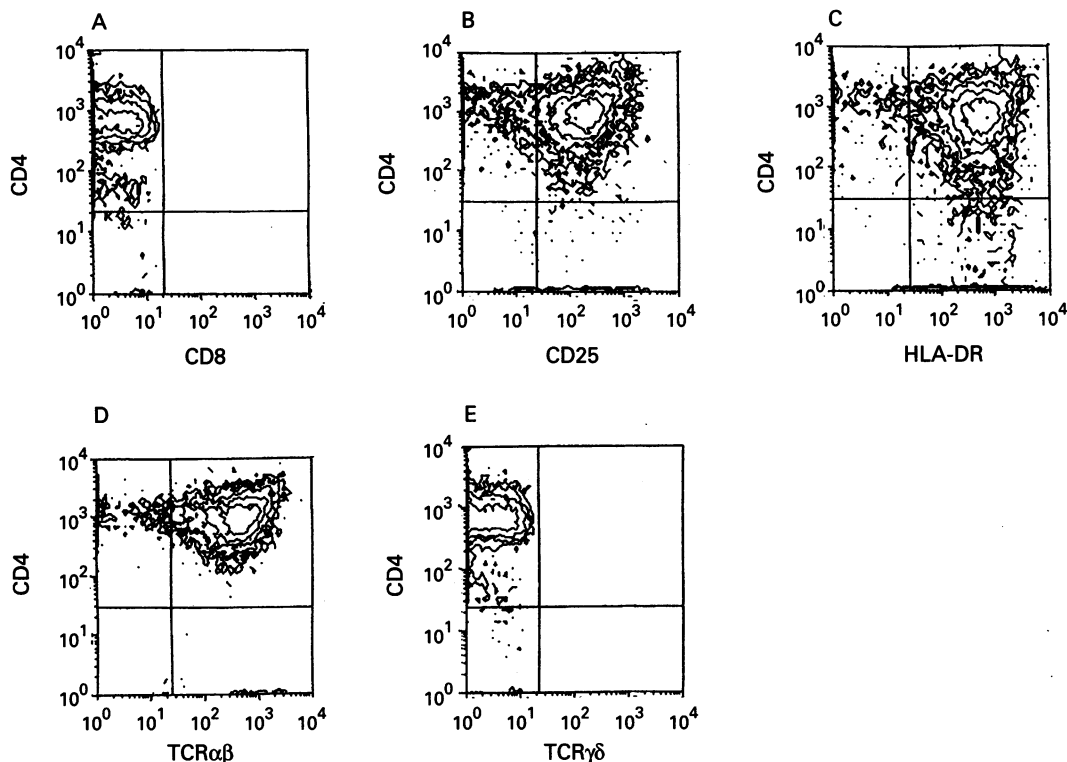


Figure 3 Flow cytometric analysis of a representative SF cell line (SF-1). SF-1 cells were stained with FITC conjugated anti-CD4 MAb (A, B, C, D, E), PE conjugated anti-CD8 MAb (A), PE conjugated anti-CD25 MAb (B), PE-conjugated anti-HLA-DR MAb (C), PE conjugated anti-TCR $\alpha\beta$ MAb (D), and PE conjugated anti-TCR $\gamma\delta$ MAb (E).

Table 1 Cytokine production and sIL-2R secretion by P-36 specific CD4⁺CD8⁻ CTL lines derived from two patients with Vogt-Koyanagi-Harada disease

Cell line	P-36 stimulation	IFN- γ (pg/ml)	IL-2 (pg/ml)	IL-4 (pg/ml)	IL-6 (pg/ml)	sIL-2R (U/ml)
SF (n=3)	-	380 (60)	17.8 (14.3)	< 3.0	554.9 (92.3)	241.3 (93.7)
	+	456 (226)	12.6 (15.7)	< 3.0	2719.4 (441.1)*	1314.0 (288.3)*
Fresh serum (patient SF)		< 15.0	15.9	< 3.0	5.7	341.0
TS (n=3)	-	320 (48)	11.5 (4.4)	< 3.0	756.2 (64.1)	482.2 (55.9)
	+	540 (82)	10.1 (5.9)	< 3.0	2461.0 (394.6)*	1579.0 (382.4)*
Fresh serum (patient TS)		< 15.0	0.0	< 3.0	6.9	281.0
Normal control sera		< 15.0 (n=16)	< 5.0 (n=20)	< 3.0 (n=40)	< 5.0 (n=36)	145-519 (n=275)

Supernatants of SF line cells (1.0×10^6 cells/ml) were harvested on the third day of culture with or without antigen stimulation. Data were shown as mean (SD).

* Indicates $p < 0.02$ compared with the cytokine production or sIL-2R secretion of CTL lines without P-36 stimulation.

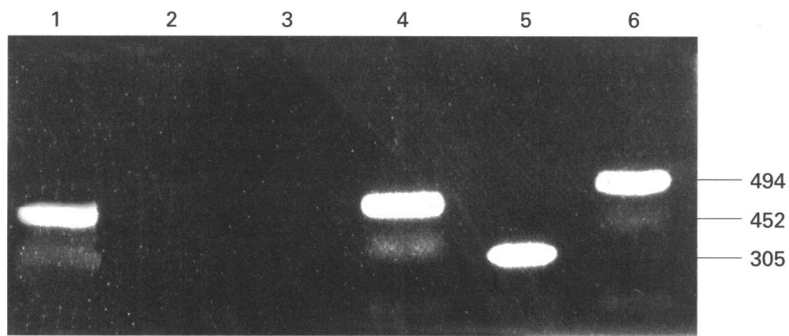


Figure 4 RT-PCR analysis of cytokine mRNA of SF-1 cells. Lanes 1 to 3, GAPDH, IL-2, and IFN- γ mRNAs of P-36, respectively; lanes 4 to 6, GAPDH, IL-2, and IFN- γ mRNAs of SF-1 cells after 24 hours incubation with P-36, respectively. The molecular sizes of PCR products of IL-2, GAPDH, and IFN- γ were 305, 452, and 494 base pairs, respectively.

lines produced high levels of IFN- γ , IL-2, and sIL-2R, but low levels of IL-4.

The abundance of transcripts encoding IFN- γ and IL-2 in SF-1, as a representative cell line, was measured by RT-PCR 24 hours after coculture with P-36 (Fig 4). IFN- γ mRNA and IL-2 mRNA were detected in the cell line, but not in P-36.

ELISA of the culture supernatant of P-36 and RT-PCR analysis of the IL-6 mRNA level of cultured P-36 have shown that P-36 does not secrete IL-6 (Norose *et al*, unpublished data).

The production of IFN- γ , IL-6, sIL-2R determined by ELISA, and IL-2 mRNA and IFN- γ mRNA determined by RT-PCR in these CTL lines suggest that these cell lines may represent human Th1 cells.

Discussion

In general, the helper/inducer function is associated with the CD4⁺CD8⁻ subset restricted by the major histocompatibility complex (MHC) class II antigens and the cytotoxic/suppressor function is associated with the CD4⁻CD8⁺ subset restricted by MHC class I antigens. However, exceptions to this pattern have been observed, such as induction of cytotoxicity by MHC class II restricted CD4⁺ CTLs in some viral and protozoan infections and autoimmune diseases.⁹⁻¹⁵ Furthermore, both melanoma specific CD8⁺ CTLs restricted by MHC class I (HLA-A2)¹⁶⁻²² and other CD4⁺ CTLs restricted by MHC class II molecules have been reported.²³⁻²⁶

Uveoretinitis induced by retinal S-antigen in rats is mediated by CD4⁺ MHC class II restricted helper/inducer T cells.^{27,28} McClellan *et al*²⁹ isolated CD4⁺ CTLs from patients with VKH that had antigen specificity in both cytotoxic and proliferative assays to cultured normal melanocytes and to allogeneic melanoma cells. An immunohistochemical study² has shown that the number of CD4⁺ lymphocytes in the choroid of patients with VKH is greater than the number of CD8⁺ cells. We previously found that the infiltrating cells in the aqueous humour of patients with acute phase endogenous uveitis (including VKH) were primarily CD4⁺ HLA-DR⁺ lymphocytes.³⁰

These findings suggest that CD4⁺ CTLs predominate in the initial immunopathological responses in VKH.

The supernatants from T cell lines cultured with or without P-36 showed no cytotoxic activity against P-36 in the present study (data not shown). The cytotoxic activity of CD4⁺ CTLs has already been shown to be specific for P-36 as previously reported.³¹ Furthermore, the blocking effect of the anti-HLA-DR MAb was observed at 6 hour assay culture of effector (CTL) cells. These data strongly suggest that target P-36 cells were killed by CD4⁺ CTLs or the lines via direct contact but not via factor mediated or bystander killing. Mauri *et al*³² recently reported that MHC class II⁺ T cell blasts or allogeneic T cell blasts preferentially induce the development of antigen specific MHC class II restricted CD4⁺ cytotoxic effector cells. HLA-DR⁺ CTL may act as antigen presenting cells. Thus, the anti-HLA-DR MAb may block such T cell antigen presenting T cell interaction as well as Th1 CTL P-36 interaction. Further experiments using a specific anti-HLA-DR4 MAb and a specific-HLA-DR1 MAb to discriminate these possibilities are needed. We would have included an anti-HLA-DR4 MAb and an anti-HLA-DR1 MAb in the present experiments if they had been available.

Our previous study showed that the cytotoxic activity of PBLs from patients with VKH against ⁵¹Cr labelled P-36 target cells was not inhibited when the unlabelled human B cell line EBV-Wa (HLA phenotypes: HLA-A24, Bw54, DR4) was added to the assay culture.³¹ This suggests that CTLs induced in patients with VKH recognise the melanoma antigen presented by HLA-DR4 molecules of P-36 but not HLA-DR4 molecules on EBV-B lymphoma (EBV-Wa). S-antigen and interphotoreceptor retinoid binding protein (IRBP) have been shown to be antigenic molecules in patients with VKH.³³ If cultured human melanoma cells and normal melanocytes have common antigenic determinants or antigenically cross reactive components on their cell surfaces, then CTLs originally generated in patients with VKH would recognise such melanoma cells in vitro. P-36 has been shown to share surface antigens with normal adult melanocytes.³⁴ Whether CTLs recognise a melanocyte specific antigen, and not a tumour antigen, has not been proved. Evidence that CTLs kill DR matched melanocytes or melanoma cells would provide confirmation of HLA-DR restriction of CD4⁺ CTLs. Further studies are needed to determine the amino acid sequence of T cell epitopes bound to HLA-DR4 molecules of P-36. Hirose *et al*³⁵ established a cell line and clones of lymphocytes from a healthy donor, with specificity to S-antigen. The need for distinctive culture conditions in which round bottom wells, high concentrations of antigen and extended incubation periods are used suggests that the cells which specifically respond towards S-antigen have low affinity and that these cells constitute a small proportion of PBLs in healthy donors. In this study, P-36 specific HLA-DR restricted CD4⁺ CTL lines were not

established from the PBLs of HLA-DR4 matched healthy volunteers and HLA-DR4 unmatched patients with VKH (unpublished data).

Lymphokine activated killer (LAK) cells can be isolated as tumour specific killer cells from a tumour bearing host after selective expansion in vitro by culture in IL-2. We previously found no significant NK cell activity in PBLs from patients with VKH.³ The possibility that the CTLs used in the present study were LAK cells is ruled out for the following reasons: the cytotoxic activity of the CTLs to P-36 was blocked by MAbs specific for HLA-DR molecules in the present study; the CTLs have been cultured for more than 3 to 4 months, in contrast with the short term survival of LAK cells³⁶; and the surface markers of the CTLs are CD16⁺CD56⁻. Most LAK cells are believed to be NK cells and CD16 and CD56 molecules are believed to be the surface antigens of NK cells.

IFN- γ is a potent multiple regulatory mediator of immune responses. Sakamoto *et al*² detected the HLA-DR antigen, which was not present in normal choroidal melanocytes, in choroidal melanocytes obtained from patients with VKH, suggesting that HLA-DR antigen expression is important in the initiation and/or perpetuation of immune reactivity in VKH. IFN- γ induced not only HLA class I but also class II expression on human uveal melanoma cells in vitro.³⁷ Upregulation of HLA-DR mRNA levels in P-36 cells induced by IFN- γ was also confirmed by RT-PCR.³⁸ IFN- γ released by CD4⁺ CTLs in the inflammatory uvea in patients with VKH may induce expression of HLA-DR antigen on melanocytes, rendering them susceptible to CD4⁺ CTLs. These findings suggest that IFN- γ may be a significant contributor to the immunopathophysiology of VKH. The role of HLA class II antigens (such as HLA-DR4) in the production of cytokines that activate particular T cell subsets in VKH remains to be clarified.

In our previous study,¹ no significant IL-2 production was observed in aqueous humour, serum, or cerebrospinal fluid obtained from patients with VKH. In the present study, the CTLs produced low levels of IL-2 even though they were stimulated by P-36 in vitro and a high level of secretion of sIL-2R and a high density of CD25 molecules on the cell surfaces of the CTLs were detected during culture. The CTLs showed, however, a high level of IL-2 mRNA when they were stimulated by P-36 in vitro.

A further investigation of other mechanisms of cytotoxicity in the immunopathogenesis of VKH and further study of a comparison of cell lines from other individuals with VKH and from individuals with other types of uveitis are needed.

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