Investigative Ophthalmology & Visual Science, Vol. 30, No. 1, January 1989

Class II Antigens on Retinal Vascular Endothelium, Pericytes, Macrophages, and Lymphocytes of the Rat

Leslie S. Fujikawa, Collin Reay, and Mark E. Morin

Class II histocompatibility complex antigens on the retinal vascular endothelium may allow these cells to function as antigen-presenting cells to circulating T cells. The present study investigated induction of class II antigens in vitro to characterize the response under controlled conditions. Retinal vascular endothelium from Lewis and Brown Norway rats (high versus low responders in experimental autoimmune uveitis) were exposed in vitro to recombinant rat gamma interferon, interleukin-1, interleukin-2, or Concanavalin-A spleen supernatant. Retinal pericytes, macrophages and lymphocytes were studied in comparison. A newly adapted ELISA technique was used to assay levels of antigen expression. Class II antigens (I-A OX6, I-E OX17, polymorphic I-A OX3), class I antigens (OX18), macrophage marker (OX42), macrophage and T helper cell marker (W3/25), and T suppressor/cytotoxic cell marker (OX8) were studied. Results showed that retinal vascular endothelium normally expresses very little class II antigen. However, high levels of I-A and I-E were induced by interferon or spleen supernatant. The levels of class II antigen approached that of the traditional antigen-presenting cell (macrophage) and were much higher than levels for pericytes and lymphocytes. The same doses of interferon showed larger increases in the Lewis rat compared to Brown Norway. No effect was seen with interleukin-1 or -2. Therefore, retinal vascular endothelium may be induced by gamma interferon to express class II antigens with degree of induction greater than or equal to the macrophage, and higher levels of induction were seen in the high responder strain. Results suggest that the retinal vascular endothelium plays an active role in the local immune response in uveitis, and levels of class II antigen induction may determine susceptibility to uveitis. Invest Ophthalmol Vis Sci 30:66–73, 1989

The essential role of the class II major histocompatibility complex antigens in the immune response has been well documented.[1-3] Class II antigens on the surface of the accessory cell (antigen presenting cell) are necessary for the triggering of the immune response.[1-3] Interaction between the class II molecule, foreign protein antigen, and the T cell antigen receptor is a necessary first step in the initiation of the immune response.[1-3] Without the expression of the class II molecule, this interaction does not occur, and the immune response is not triggered.[1,2]

It is also clear that cells other than those traditionally thought of as antigen presenting cells may be induced to express class II antigens and can therefore be active participants in the development of the cell-mediated immune response.[3-6] Vascular endothelial cells, for example, have been shown to express class II antigens in the disease experimental autoimmune uveitis (EAU).[5,6] We have previously shown with immunohistochemical evidence that retinal vascular endothelial cells begin to express class II antigens just prior to clinical onset of uveitis (EAU),[5,6] consistent with a role for these cells in the initiation of EAU. Whether this sudden induction of class II expression is the primary cause of the monocellular infiltration into the retina characteristic of experimental uveitis is not known.

In the present study we examined more quantitatively the expression of class II antigens (I-A and I-E) on rat retinal vascular endothelial cells in vitro, comparing these cells to the rat macrophage (traditional antigen presenting cell), lymphocytes, and to local pericytes from retinal vasculature, using a reliable enzyme-linked immunoabsorbant assay (ELISA) that was developed in this laboratory. We demonstrate large increases in class II antigen expression on retinal vascular endothelium upon exposure to gamma interferon, at levels comparable to those of the macrophage. In addition, we show differences in class II

From the Ocular Immunology Laboratory, Smith-Kettlewell Eye Research Foundation, Pacific Presbyterian Medical Center, San Francisco, California.

Supported by NIH grant EY-06948 (LSF), Bethesda, Maryland, the Smith-Kettlewell Eye Research Foundation, San Francisco, California, Research to Prevent Blindness, Inc., New York, New York, and Pacific Presbyterian Medical Center, San Francisco, California.

Submitted for publication: December 17, 1987; accepted July 27, 1988.

Reprint requests: Leslie S. Fujikawa, MD, Director, Ocular Immunology/Uveitis, Smith-Kettlewell Eye Research Foundation, Pacific Presbyterian Medical Center, 2232 Webster Street, San Francisco, CA 94115.

induction in Lewis versus Brown Norway rat, which may be of great importance in determining susceptibility to uveitis.

Materials and Methods

Animals

Lewis and Brown Norway rats (35–50 g) were used as the source of retinal vascular endothelium, pericytes, macrophages, and lymphocytes. All rats were sacrificed by CO_2 asphyxiation. Tissues and cells were harvested immediately and isolated separately. The use of animals in this study conformed to the ARVO Resolution on the Use of Animals in Research.

Culture of Retinal Vascular Endothelium

Retinal vascular endothelium was cultured as described previously.[7] Briefly, retinas were removed and digested in 3 mg/ml collagenase (Type II, Worthington Biochemical, Freehold, NJ). Graded filtration was used to isolate microvessels, which were plated onto collagen-coated dishes. Confluent monolayers of endothelium were obtained and used as source of retinal vascular endothelium within 1–2 months of primary culture. Cells were identified by characteristic morphology and uptake of di-I-acetylated low-density lipoprotein (LDL),[8] and were greater than 99% pure endothelial cells by these criteria.

Culture of Retinal Pericytes

Pericytes were cultured from retinal microvessels using similar initial techniques as for retinal vascular endothelium. Growth of retinal pericytes was enhanced in medium containing lymphokine supernatants, and pericytes were separated from endothelial cells by weeding and serial passage as described previously.[9] Cells were identified by distinctive morphology and by lack of uptake of di-I-acetylated LDL,[8,9] and were greater than 99% pure pericytes by these criteria.

Isolation of Macrophages

Sacrificed rats were disinfected around the abdomen with 70% ethanol. Skin was reflected back, using a midline incision, exposing the peritoneal cavity. Using a syringe, 20 cc of Hanks balanced salt solution with 100 units/ml of heparin added was injected into the abdominal cavity. The fluid-filled cavity was then agitated in order to suspend free macrophages in the solution. The fluid was then withdrawn and centrifuged for 10 min. The supernatant was drawn off, and the cells were resuspended in cell medium consisting of M-199 plus 1% penicillin-streptomycin (100 units/ml penicillin and 100 μg/ml streptomycin) and 10% fetal calf serum (FCS).

Preparation of Lymphocytes

Mesenteric lymph nodes were removed after macrophages were harvested. Cells were teased from lymph nodes with forceps and passed over a nylon wool column to remove adherent cells (macrophages). Nylon wool columns are a standard method of enriching for T cells, and the eluted population was comprised of greater than 99% lymphocytes by morphology and immunohistochemical staining (T cells positive for W3/25 and OX8). Cells were resuspended in medium consisting of RPMI 1640 plus 1% penicillin-streptomycin and 10% FCS. Alternatively, lymphocytes were cultured in M-199 plus 1% penicillin-streptomycin and 10% FCS (macrophage medium). No differences were noted in cell morphology or staining characteristics between these two media.

Lymphokine Preparations

Concanavalin A (Con A) stimulation of rat splenocytes was achieved, and culture supernatants used as source of lymphokines, termed "lymphokine supernatants." Splenocytes (5×10^6/ml) were incubated with 5 μg/ml Con A, after lysing erythrocytes with ammonium chloride lysing buffer. Culture medium consists of RPMI 1640 with 5×10^{-5} M 2-mercaptoethanol, 2% glutamine, 1% penicillin-streptomycin and 1% syngeneic rat serum. After 40 hr, supernatants were collected, passed through 0.22 μm filter and stored in aliquots at −20°C. Con A was inactivated prior to use with 0.1 M alpha-methyl mannoside. Such activated supernatants contain many substances, including a host of lymphokines. Supernatants were tested for induction of class II antigens on cultured endothelium and macrophages in Lab-Tek slides (Miles Scientific, Naperville, IL) as described previously.[7] The highest dilution of supernatant which induced maximal expression of class II antigens on both cell types was selected for use in the present ELISA experiments (1:8 for Brown Norway supernatant; 1:16 for Lewis supernatant).

Recombinant rat gamma interferon was obtained from AMGen Biologicals (Thousand Oaks, CA), and was diluted to a stock solution of 1×10^5 units/ml. Appropriate dilutions were made in medium prior to addition of gamma interferon to the cultures. Interleukin-1 (IL-1) (Collaborative Research, Bedford, MA [human interleukin-1, CR-hIL 1]) was diluted to 10 half-maximal units/ml with appropriate medium, a concentration 2 to 3 times that required to promote maximum ^{3}H-thymidine incorporation by normal

mouse thymocytes.[10] Rat interleukin-2 (IL-2) (Collaborative Research [CR-rIL 2]) was also diluted to 10 half-maximal units/ml with appropriate medium. This level of IL-2 will fully stimulate the proliferation of most responsive T cell types.[11]

Preparation of ELISA Plates

Endothelial cells and pericytes were adjusted to 3 $\times 10^4$ cells/ml in growth media (see above) after trypsinization. Macrophages were resuspended in M-199 plus 1% penicillin-streptomycin and 10% FCS at 1.0 $\times 10^6$ cells/ml. Lymphocytes were resuspended in medium consisting of M-199 plus 1% penicillin-streptomycin and 10% FCS or RPMI 1640 plus 1% penicillin-streptomycin and 10% FCS, each at 1.0 $\times 10^6$ cells/ml. No differences in ELISA results were seen for lymphocytes grown in these two media. Cell suspension (0.2 ml) was pipetted into each well of a 96-well plate coated with collagen. Lymphocyte cultures were plated into wells coated with poly-L-lysine to which they adhere more readily than collagen. Outer wells were not used in order to avoid artifacts of the edge effect: inconsistent optical density (O.D.) readings seen from wells along the periphery of the plate. Endothelial cells, macrophages, and pericytes were then incubated for 3 days at 37°C in a 5% CO_2 humidified incubator. On day 3, each well received fresh media. Additives containing various lymphokine preparations were placed into the even rows. These rows of wells were the treated wells. Plates were then reincubated for an additional 4 days at 37°C. Lymphocyte cultures received additives on day 1 and were incubated for an additional 4 days. On the final day, media was aspirated gently so as not to disturb cells, and wells were dried at room temperature for 30–60 min. After complete drying, plates were either used immediately or frozen at −20°C until assayed. Using this procedure, cells remained adherent and stable for ELISA assay for up to 2 months after initial drying. Loss of cells from the wells during medium aspiration and assay was minimal under the conditions used.

Monoclonal Antibodies against Rat Determinants

Antibodies against class II antigens were obtained from Accurate Chemical & Scientific Corp. (Westbury, NY). OX6 (ascites, 1:200) is directed against nonpolymorphic class II antigen I-A. OX17 (supernatant, 1:8) is directed against nonpolymorphic class II antigen I-E. OX3 (supernatant 1:5) is directed against polymorphic class II antigen and is specific for the Lewis rat[12] in our experiments. OX18 (supernatant 1:8) is directed against nonpolymorphic class I antigen. OX42 (ascites 1:100) was obtained from Bioproducts for Science, Inc. (Indianapolis, IN) and is a known marker for macrophages. W3/25 (supernatant 1:8 from Accurate) is known marker for T helper cells and macrophages (13). OX8 (supernatant 1:8 from Accurate) is known marker for T suppressor/cytotoxic cells.

ELISA

Wells were first rinsed twice using ELISA wash solution, consisting of 0.01 M phosphate buffered saline (PBS), 0.05% Tween-20™ (Sigma Chemical Co., St. Louis, MO), and 0.01% Thimerosal (Sigma). For all washes approximately 200–250 μl of wash solution was gently pipetted into each well and then flicked out. In order to reduce nonspecific binding of antibody, plates were incubated with 200 μl of 20% or 50% normal goat serum (lot 1 versus lot 2), followed by 50 μl of 50% normal rat serum, each for 30 min at 37°C. Primary antibody (50 μl) was then delivered to each well (50 μl of appropriate dilutions of OX3, OX6, OX17, OX18, OX8, W3/25 from Accurate, or OX42 from Bioproducts for Science, Inc.). All antibodies were diluted with the ELISA wash solution. Plates were incubated for 60 min at room temperature. After incubation plates were washed three times with the ELISA wash solution allowing a 60 sec incubation period for the first wash. The secondary antibody, 50 μl of biotinylated rat anti-mouse IgG (1:100) (Accurate), was then added to each of the wells and the plate incubated at room temperature for 30 min. After incubation the plate was again washed three times with ELISA wash, allowing a 60 sec incubation for the first wash. Next, 50 μl of a streptavidin peroxidase conjugate (1:1000 dilution) was delivered to each well and the plate incubated at 37°C for 10 min. The plate was again washed three times, after which 100 μl of freshly made substrate solution was delivered to each well. Substrate solution consisted of 0.1 M citrate phosphate buffer, 0.05% H_2O_2, and 0.035% 2–2'-azino-di-(3 ethyl-benzothiazoline sulfonic acid) diammonium salt. After 30 min incubation at room temperature, the optical density was read at 405 nm. Results are expressed as the mean of replicate measurements with two standard deviations shown. A total of six measurements were usually averaged (three separate experiments with duplicates for each treatment group).

Results

Rat Macrophages

Significant binding of primary antibodies OX42 and W3/25 directed against known markers on the rat macrophage was observed in comparison to the controls (Fig. 1A). In all assays controls consisted of populated wells that received no primary antibody. Control wells from all experimental runs showed little variation in optical densities. The lack of T cell

contamination was shown by the very low binding of OX8.

Low levels of binding of antibodies directed against separate class II histocompatibility complex antigens was observed in comparison to the controls, as shown in Figure 1B (untreated OX6 and OX17). Both I-A (OX6) and I-E (OX17) were expressed at consistently low, but detectable levels.

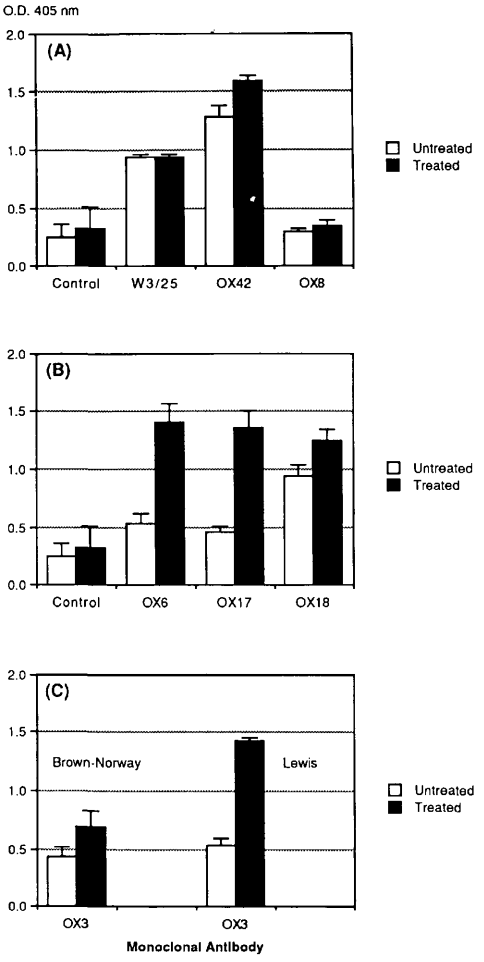

Fig. 1. ELISA of rat macrophages. Normal, untreated macrophage cultures are compared with cultures treated with lymphokine supernatant. Brackets enclosed two standard deviations. (**A**) W3/25 and OX42 are known macrophage markers and react positively in this assay. OX8 is the marker for T cells of suppressor/cytotoxic phenotype, and it is negative on rat macrophages. Controls are stained with secondary antibody only. (**B**) Increased class II antigen expression upon treatment with lymphokine supernatant is demonstrated for OX6 and OX17. OX18 demonstrates class I antigens and shows a slight increase upon treatment. (**C**) OX3 is a polymorphic reagent, specific for class II molecule of the Lewis rat and not for those of the Brown-Norway rat. Cultures are Brown-Norway macrophages, except where noted, and Brown-Norway lymphokine supernatant is used at 1:8 dilution.

Table 1. Immunohistochemical staining of rat macrophages

Monoclonal antibody	*Percent positive cells**	
	Normal medium	*Medium plus lymphokine sup.*
OX6	10.3 (20/194)	96.0 (184/192)
OX17	5.8 (11/187)	95.5 (191/200)
OX18	53.0 (97/183)	98.1 (156/159)
Control†	0.0 (0/200)	12.07 (21/174)

* Percent positive cells were calculated from counting darkly stained cells within photographs of individually stained cultures. Approximately 200 cells per culture were counted, and results are expressed as % positive = # darkly stained − total # counted ×100.

† Secondary antibody only.

Upon treatment with Concanavalin-A-induced spleen cell supernatants, there was a striking increase in expression of both class II antigens, I-A and I-E (treated OX6 and OX17, respectively) (Fig. 1B). This three-fold increase was consistent and comparable for the two class II antigens. OX3, the marker for Lewis class II antigens, was seen to increase in the Lewis rat and not in the Brown Norway (Fig. 1C).

There was a small increase in class I expression (33%), as demonstrated by OX18 (Fig. 1B). OX42, OX8 and controls showed no significant increase upon treatment with lymphokine-containing supernatants (Fig. 1A).

These results compared favorably with immunohistochemical evidence. Immunoperoxidase staining of the rat macrophage (Brown Norway and Lewis) class II antigens (OX6 and OX17) showed less than 11% staining when grown without supernatant and greater than 95% staining frequency when cells were incubated in supernatant (Table 1). ELISA results compare very favorably with a three fold increase in OX6 and OX17 binding. Immunoperoxidase staining of class I antigen (OX18) showed 53% of macrophages stained when untreated and 98% stained when they received treatment with lymphokine-containing supernatant. This ELISA indicated a 33% increase in OX18 binding when the rat macrophages were treated.

Retinal Vascular Endothelium and Pericytes

Treated retinal vascular endothelial cells and pericytes were measured for class I and II antigen expression. The retinal vascular endothelium demonstrated a large increase in class II antigen expression upon treatment with lymphokine supernatant when compared with untreated endothelial cells or controls

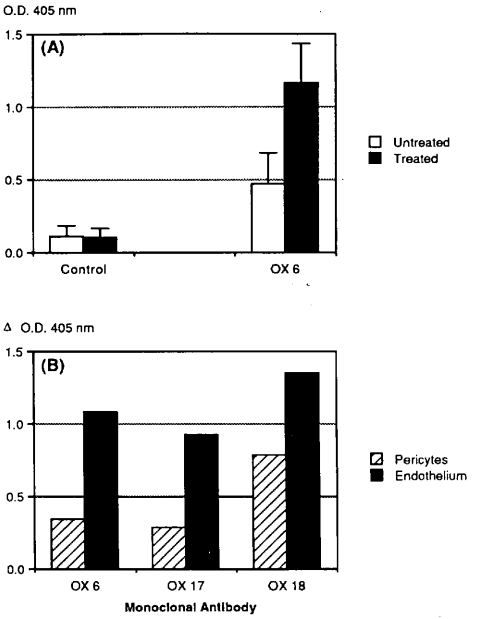

Fig. 2. Induction of class II antigens on the retinal vascular endothelium. **(A)** Retinal vascular endothelium treated with lymphokine supernatants demonstrates high levels of class II antigens (OX6) when compared with untreated cultures and control cultures. **(B)** Treated retinal vascular endothelium also demonstrates very high levels when compared with treated retinal pericytes. Controls are incubated with secondary antibody only. Results for **(B)** are expressed as ΔO.D. 405 nm = treated minus untreated values. Cultures are Lewis retinal vascular endothelium and Lewis lymphokine supernatant 1:16.

(Fig. 2A). Treated pericytes were observed to exhibit lower levels of class II antigens (OX6 and OX17), in comparison to retinal vascular endothelium and to class I antigens (OX18) (Fig. 2B).

Retinal vascular endothelial cells, in contrast, exhibited much higher levels than pericytes of both class II antigens, I-A and I-E (OX6 and OX17), and levels approached those seen for class I antigen (OX18) on retinal vascular endothelium (Fig. 2B). ELISA of retinal vascular endothelium also yielded increases in levels of class II antigens approximating those of the traditional antigen-presenting cell, the macrophage (OX6 ΔO.D. 405 = 1.079 for endothelium and 0.878 for macrophage).

Varying Concentrations of Supernatant

When two concentrations of Brown Norway supernatant were compared in macrophages, the results indicated that there was a higher level of both class I and class II expression when a higher concentration of supernatant was used. The results summarized in Table 2 show higher levels of expression of OX6, OX17, and OX18 at the higher concentration of supernatant and lower levels of expression of both class II and class I antigens at the lower concentration of supernatant. Larger differences were seen for OX6 and OX17 than for OX18.

Lewis supernatant consistently induced higher levels of class II antigens at a lower concentration of supernatant compared to Brown Norway supernatant: O.D. 405 nm 1.379 (SD = 0.248) for Lewis supernatant concentration of 1:16 versus 1.266 (SD = 0.298) for Brown Norway supernatant at 1:8.

Effects of Purified Lymphokines

Recombinant gamma interferon led to a striking increase in class II antigen expression on retinal vascular endothelium at levels comparable to that of the macrophage (Fig. 3). Nylon-wool-purified lymphocytes, in contrast, demonstrated only a small change in class II antigen expression (Fig. 3). Levels of class II antigen induction were slightly higher on lymphocytes when grown in the presence of Con-A supernatant (OX6 ΔO.D. 405 nm = 0.167 in supernatant versus 0.052 in recombinant gamma interferon; O.D. 405 nm = 0.599 (SD = 0.117) for untreated cultures), but levels were still far below those on retinal vascular endothelium or the macrophage.

The increase in class II antigen upon addition of lymphokine was specific to gamma interferon and the Con-A lymphokine supernatant. Purified interleukin-1 (10 units/ml) and interleukin-2 (10 units/ml) were added to the rat cultures and found to have no effect on the expression of class I or II antigens (Fig. 4).

Table 2. Class II antigen induction with varying concentrations of supernatant

Monoclonal antibody	*O.D. 405 nm*		
	Untreated	*Treated 1:16 sup.*	*Treated 1:8 sup.*
OX6	0.562 (SD = 0.001)	1.045 (SD = 0.027)	1.463 (SD = 0.078)
OX17	0.532 (SD = 0.007)	1.172 (SD = 0.006)	1.309 (SD = 0.047)
OX18	0.895 (SD = 0.004)	0.916 (SD = 0.015)	1.133 (SD = 0.077)
OX3	0.514 (SD = 0.018)	0.662 (SD = 0.136)	0.825 (SD = 0.018)

Class II Induction in Lewis versus Brown Norway Rat

Known concentrations of recombinant rat gamma interferon were added to the rat cultures, and doses of between 0.2 and 2000 units/ml demonstrated increasing class II antigen expression which varied with the $\log_{10}$ concentration of gamma interferon (Fig. 5). Recombinant gamma interferon at a concentration of 20 units/ml was sufficient to maximally induce class II antigens on both Brown Norway and Lewis macrophages (Fig. 5). Higher levels and larger increases were seen for the Lewis macrophages than for Brown Norway (compare Figs. 5A and B for Lewis and 3D and E for Brown Norway). Doses of between 0.02 and 200 units/ml were used for Lewis versus doses of between 0.2 and 2000 units/ml for Brown Norway. Class I antigens were also increased by addition of gamma interferon, although the change was less because of high baseline levels of class I antigen expression in both Lewis and Brown Norway (Fig. 5C, F). Lewis cultures demonstrated slightly larger increases in class I antigen expression (Fig. 5C), when compared to Brown Norway (Fig. 5F).

Discussion

In the current study, we demonstrate that retinal vascular endothelial cells in vitro experienced a large increase in expression of class II antigens, both I-A and I-E, upon exposure to recombinant rat gamma interferon or lymphokine spleen supernatants containing gamma interferon. Levels of class II antigen induction approached or exceeded those of the traditional antigen-presenting cell, the macrophage, and were far greater than low levels on retinal pericytes: levels of class II antigen induction (difference in O.D. 405 nm readings between treated and untreated cultures) were equivalent in endothelial cultures when compared to cultures of macrophages, despite lower numbers of endothelial cells seeded per culture, possibly indicating higher levels of class II antigen per cell for endothelium than for the macrophage; low levels of class II induction seen on the retinal pericyte are consistent with its lack of known role in the immune response. Rat lymphocytes also showed very little class II antigen induction under the conditions tested.

The expression of class II antigens on cell surfaces promotes those cells into becoming active participants in the development of a local immune response.[3,4] This is a function thought normally to be mediated by the antigen-presenting cell, eg, macrophage, which internalizes and processes foreign antigens and then re-expresses them on its surface in conjunction with the class II histocompatibility complex molecule.[1-4] The class II molecule, when coexpressed with the processed antigen on the surface of the antigen-presenting cell, activates the T lymphocyte.[1,2] This stimulation of the T cell leads to a series of immunological events, including lymphokine production with local recruitment of inflammatory cells.[1-4] In this way, localized inflammation is established and perpetuated.

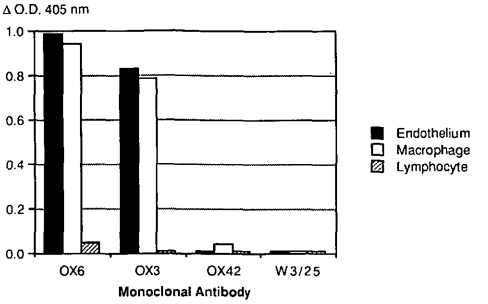

Fig. 3. Comparison of class II expression of retinal vascular endothelium (Endothelium), macrophages, and lymphocytes. Retinal vascular endothelium is induced to express levels of class II antigens comparable to that of the macrophage. Results are expressed as ΔO.D. 405 nm = treated minus untreated values. Treatment consisted of incubating Lewis cultures in medium containing 1000 units/ml of gamma interferon.

It has become clear that retinal vascular endothelial cells may participate actively in the inflammation typically seen in uveitis.[5,6] In vivo, we have shown that class II antigens are expressed on the retinal vascular endothelium during experimental autoimmune uveitis (EAU),[5,6] as well as during human disease demonstrated on retinochoroidal biopsies from uveitis patients,[14] and upon systemic injection of

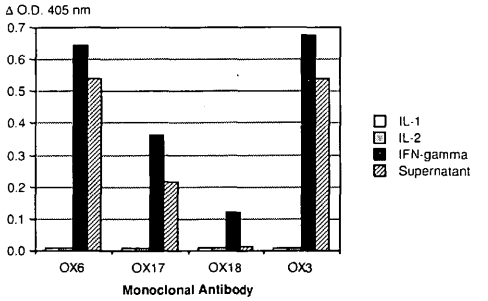

Fig. 4. Effect of purified lymphokines on class II antigen expression. Interleukin-1 (IL-1) at 10 half-maximal units/ml and interleukin-2 (IL-2) at 10 half-maximal units/ml were compared to gamma interferon (1000 units/ml) and lymphokine supernatant (Lewis 1:16) for induction of class II antigens on Lewis macrophages. Results are expressed as ΔO.D. 405 nm = treated minus untreated values. Data demonstrate large increases in class II antigen expression upon treatment with recombinant gamma interferon or lymphokine supernatant, and no changes upon incubation with IL-1 or IL-2.

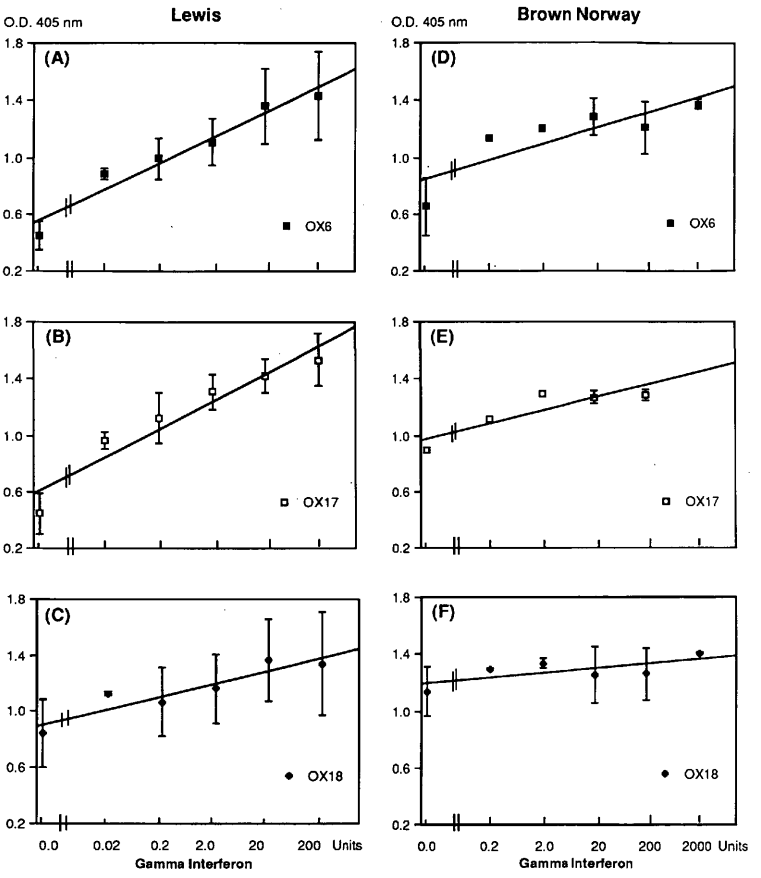

Fig. 5. Recombinant gamma interferon induction of class II antigens. Figures (A) through (C) demonstrate dose response curves for class II induction (OX6, OX17, and OX3) on Lewis macrophages by gamma interferon. Dosages from 0.02 to 200 units/ml of rat gamma interferon demonstrated increasing class II antigen expression which varied with the $\log_{10}$ concentration of gamma interferon. Figures (D) through (F) demonstrate dose-response curves for class II induction (OX6, OX17) and class I expression (OX18) on Brown-Norway macrophages by gamma interferon. Increases were not as dramatic as for Lewis macrophages with dosages from 0.2 to 2000 units/ml of rat gamma interferon used.

gamma interferon.[15] These results indicate that these cells may function as antigen presenting cells during uveitis, leading to the local immune response in the eye. Our current data indicate that the retinal vascular endothelium strongly expresses class II antigens at levels comparable to that of the macrophage, and therefore may be an integral part of the immunopathologic sequence that leads towards establishing this disease.

The class II histocompatibility complex antigen is in itself a key regulator in the immune response, and recent studies indicate that the molecular configuration of the class II molecule may determine its ability to bind antigen and thereby control immune responsiveness.[1,16] One of the functions of the class II molecule may be to bind to some, but not all, peptide antigens created during antigen processing and determine the antigen to be presented to the T cell. Thus, it has been shown that class II molecules from high responder strains bind directly to specific immunogenic antigen, whereas class II molecules from low responder strains do not bind to that antigen.[17,18]

This correlation between antigen-class II interaction and disease susceptibility may have great importance in our studies of uveitis. For example, in experimental autoimmune uveitis, the Lewis rat is a high responder, whereas the Brown Norway rat is a low responder.[19] It may be that the class II molecule expressed on the surface of the retinal vascular endothelium from Lewis rat binds more strongly to retinal antigens than those from the Brown Norway rat. This may help to explain on a molecular basis the immune response in uveitis.

This study demonstrates that class II induction upon treatment with the same dose of recombinant gamma interferon is different in the Lewis versus the

Brown Norway rat: higher responses and larger increases in class II antigens were seen in the Lewis rat. In addition, the lymphokine supernatants from Lewis rat generated consistently higher levels of class II inducing lymphokines than those from the Brown Norway rat (see results above). These data indicate higher sensitivity of the Lewis rat to immunologic stimuli resulting in increased class II antigen expression when compared to the Brown Norway rat. Results are consistent with our recent work in which we demonstrate that systemic administration of gamma interferon leads to much greater induction of class II antigens on the retinal vascular endothelium of the Lewis rat when compared to the Brown Norway.[15] This differential induction of class II antigens may contribute significantly to differences in immune responses among strains and may help explain susceptibility to uveitis among rats of different strains.

Current therapeutic approaches are based upon the importance of the class II antigens. Recent work in our laboratory (Fujikawa LS, unpublished data) indicates that monoclonal antibodies against class II antigens may block the induction of EAU. This new treatment is directed towards the highly specific interaction between antigen presenting cell and T cell. The above studies may help to explain immune regulation on a molecular basis stressing the essential role of the class II molecule in determining immune responsiveness in uveitis.

The induction of class II molecules on cells not traditionally thought to be part of the immune system, such as retinal vascular endothelium, occurs through the action of soluble mediators, such as gamma interferon. In the current study, recombinant gamma interferon and the lymphokine supernatants induced large increases in class II expression, consistent with previous work demonstrating gamma interferon as a potent known inducer of class II antigens,[20] and with our previous work using lymphokine supernatants to induce class II antigens in vitro.[7] Interleukin-1 and interleukin-2 did not induce detectable levels of class II antigens on the retinal vascular endothelium, and they have not been shown to be direct inducers of class II antigens in other systems.[10,11] Further investigations are ongoing in our laboratory to more closely examine the source and nature of lymphokines inducing class II antigens in order to discover the source of this disease.

Key words: uveitis, ocular immunology, experimental autoimmune uveitis, retinal vascular endothelium, class II histocompatibility complex antigens

References

1. Unanue ER and Allen PM: The basis for the immunoregulatory role of macrophages and other accessory cells. Science 236:551, 1987.
2. Schwartz RH: T lymphocyte recognition of antigen in association with gene products of the major histocompatibility complex. Ann Rev Immunol 3:237, 1985.
3. Foulis AK: Class II major histocompatibility complex and organ specific autoimmunity in man. J Pathol 150:5, 1986.
4. Roska AK, Geppert TD, and Lipsky PE: Immunoregulation by vascular endothelial cells. Immunobiology 168:470, 1984.
5. Fujikawa LS, Chan CC, McAllister C, Gery I, Hooks JJ, Detrick B, and Nussenblatt RB: Retinal vascular endothelium expresses fibronectin and class II histocompatibility complex antigens in experimental autoimmune uveitis. Cell Immunol 106:139, 1987.
6. Fujikawa LS, Chan CC, McAllister C, Gery I, Hooks JJ, Detrick B, and Nussenblatt RB: Class II histocompatibility complex antigens (I-A and I-E) in experimental autoimmune uveitis. *In* Immunology and Immunopathology of the Eye, Secchi AG, editor. Milan, Masson, in press, 1988.
7. Fujikawa LS and Hung M: Class II histocompatibility complex antigens on the retinal vascular endothelium. Invest Ophthalmol Vis Sci 28:99, 1987.
8. Voyta JC, Via DP, Butterfield CE, and Zetter BR: Identification and isolation of endothelial cells based on their increased uptake of acetylated-low density lipoprotein. J Cell Biol 99:2034, 1984.
9. Gitlin JD and D'Amore PA: Culture of retinal capillary cells using selective growth media. Microvasc Res 26:74, 1983.
10. Dinarello CA, Cannon JG, Mier JW, Bernheim HA, LoPreste G, Lynn DL, Love RN, Webb AC, Auron PE, Reuben RC, Rich A, Wolff SM, and Putney SD: Multiple biological activities of human recombinant interleukin 1. J Clin Invest 77:1734, 1986.
11. Mertelsmann R and Welte K: Human interleukin 2: Molecular biology, physiology and clinical possibilities. Immunobiology 172:400, 1986.
12. Reske K and Weitzel R: Immunologically discrete conformation isomers of I-A locus-equivalent class II molecules detected in Lewis rats. Eur J Immunol 15:1229, 1985.
13. Jeffries WA, Green JR, and Williams AF: Authentic T helper CD4 (W3/25) antigen on rat peritoneal macrophages. J Exp Med 162:117, 1985.
14. Fujikawa LS and Haugen J-P: Immunopathology of vitreous and retinochoroidal biopsy in posterior uveitis. Ophthalmology 94:136, 1987.
15. Viebahn M and Fujikawa LS: Class II histocompatibility complex antigens on vascular endothelium of the rat eye: Induction by systemic lymphokine administration. Invest Ophthalmol Vis Sci 29:268, 1988.
16. Buus S, Sette A, Colon SM, Miles C, and Grey HM: The relation between major histocompatibility complex (MHC) restriction and the capacity of Ia to bind immunogenic peptides. Science 235:1353, 1987.
17. Buus S, Colon S, Smith C, Freed JH, Miles C, and Grey HM: Interaction between a "processed" ovalbumin peptide and Ia molecules. Proc Natl Acad Sci USA 83:3968, 1986.
18. Babbitt BP, Allen PM, Matsueda G, Haber E, and Unanue ER: Binding of immunogenic peptides to Ia histocompatibility molecules. Nature 317:359, 1985.
19. Gery I, Robison WG, Shichi H, El-Saied M, Mochizuki M, Nussenblatt RB, and Williams RM: Differences in susceptibility to experimental autoimmune uveitis among rats of various strains. *In* Immunology and Immunopathology of the Eye, O'Connor GR and Chandler JW, editors. New York, Masson, 1985, pp. 242–245.
20. Steeg PS, Moore RN, Johnson HM, and Oppenheim JJ: Regulation of murine macrophage Ia antigen expression by lymphokine with immune interferon activity. J Exp Med 156:1780, 1982.