

The Critical Role of IFN- γ in Experimental Coronavirus Retinopathy

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PURPOSE. Experimental coronavirus retinopathy (ECOR) is an animal model of progressive retinal disease that is first manifest as an acute retinal inflammation followed by chronic, immune-associated retinal degeneration in genetically susceptible, BALB/c mice. In retinal degeneration-resistant CD-1 mice, only the acute infection is seen. In the present study, interferon (IFN)- γ production during ECOR was studied and its role evaluated in the clearance of infectious virus from the retina.

METHODS. BALB/c, CD-1, and IFN- γ -deficient (IFN- γ gko) mice were inoculated with the JHM strain of murine coronavirus by the intravitreal route. Mouse eyes were evaluated for infiltrating cells and major histocompatibility complex (MHC) expression by immunocytochemical staining. Isolated retinas were analyzed for IFN- γ mRNA by RT-PCR, and sera were evaluated for IFN- γ protein by ELISA assays.

RESULTS. Virus infection in BALB/c mice was associated with an increase in the incidence and levels of systemic IFN- γ . Moreover, IFN- γ mRNA was detected within the retinas of infected animals during the acute phase of the disease but was not detected in normal or mock-injected animals. IFN- γ mRNA was detected at the time of T-cell infiltration, and earlier studies have shown that this is temporally related to granzyme B gene expression and the clearance of infectious virus from the retina. Retinal IFN- γ mRNA was also associated with the upregulation of MHC class I and II molecules within the retina. When this infection occurred in IFN- γ gko mice, the virus was unchecked, and the infection led to death.

CONCLUSIONS. These studies indicate that generation of IFN- γ by cells infiltrating the retina is an essential part of an immune mechanism responsible for noncytolytic clearance of infectious virus from the retina. (*Invest Ophthalmol Vis Sci.* 2003; 44:3402-3408) DOI:10.1167/iov.02-1106

The interferons (IFNs) were discovered as antiviral molecules that inhibit a variety of viruses. Two general families of IFNs exist.¹ Type I IFNs include IFN- α , IFN- β , and IFN- ω , which are encoded by a family of more than 20 genes and share the type I cellular receptor. The type II IFN is IFN- γ , which is encoded by a single gene, has a separate cellular receptor, and is produced by T cells and NK cells. All the IFNs have overlapping pleiotropic effects on a variety of cellular functions. Studies using IFN- α/β and IFN- γ receptor knockout mice have demonstrated that both IFN systems are essential for antiviral

defense and are functionally nonredundant.² Numerous studies have demonstrated that IFN- γ is important in controlling virus infections in the central nervous system (CNS). For example, measles virus-induced encephalitis, mouse hepatitis virus (MHV)-induced demyelination, and vaccinia virus in the CNS are sensitive to interferon's antiviral activity.^{3,4} Within the eye, IFN- γ protects the retina from herpes simplex virus (HSV)-induced retinitis.⁵⁻⁷

It is becoming increasingly clear that IFN- γ triggers its antiviral actions in vivo by exerting cellular effects at multiple levels.¹ First, the interaction of IFN- γ with its receptor and the subsequent induction of a variety of genes and gene products result in the downregulation of virus replication.⁸ Alternatively, IFN- γ activates cytokine production by T cells, monocytes, and resident cells, it augments cytotoxic T lymphocyte (CTL) killing by the induction of molecules such as granzyme B or major histocompatibility complex (MHC) class I, and it enhances immune reactivity through the induction of MHC class II molecules.^{1,9-11} Recent studies focusing on viral infections of the brain and liver have delineated the concept that cytokine secretion by infiltrating CTLs may be an important alternative to CTL-mediated killing.¹⁰⁻¹² These studies highlight the distinct advantage of IFN- γ -mediated noncytolytic clearance of virus from cells that are nonrenewable. We therefore, set out to evaluate the protective role of IFN- γ in an experimental coronavirus retinopathy (ECOR) model of a persistent viral infection.^{13,14}

Murine coronavirus is a naturally occurring murine hepatitis virus. Neurotropic strains have been identified. Ocular infection of susceptible mouse strains leads to a biphasic disease that is first manifest as an acute retinal inflammation followed by a chronic, immune-associated retinal degeneration.¹⁴⁻¹⁶ During the degenerative phase of the disease, virus nucleic acid persists within the retina.¹⁷ However, infectious virus cannot be found. Targets for early infection are retinal pigment epithelial (RPE) cells, ciliary body epithelial cells, and Müller-like cells and some photoreceptors.^{18,19} The role of the immune system in the degenerative phase is supported by the identification of anti-retinal and anti-RPE cell antibodies in retinal degeneration-susceptible (BALB/c) mice.¹² In contrast, these autoantibodies are absent in retinal degeneration-resistant mice (CD-1), which demonstrate only the acute phase of the disease.¹³

Therefore, this model system consists of two separate phases. The early phase is an inflammatory retinal vasculitis that is characterized by clearance of infectious virus. This occurs in both retinal degeneration-sensitive (BALB/c) and -resistant (CD-1) mice. The late phase is retinal degeneration that is characterized by immunopathologic processes and occurs in genetically susceptible BALB/c mice. In this study, we undertook the characterization of ECOR to examine the potential contribution of IFN- γ in the clearance of infectious virus in the retina. We found that IFN- γ was produced during ECOR, and IFN- γ gene expression was detected within the retina. The presence of IFN- γ was associated with the activation of host immune response to MHV infection, and the absence of IFN- γ in IFN- γ -deficient mice was associated with a dramatic increase in viral encephalitis and death. These parameters were

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similar in retinal degeneration-sensitive (BALB/c) and -resistant (CD-1) mice. Taken together, the data define IFN- γ -associated responses as critical factors regulating virus infections within the retina.

MATERIALS AND METHODS

Virus, Animals, and Inoculation

The JHM strain of MHV was obtained from American Type Culture Collection (ATCC, Manassas, VA), and passaged five to seven times in mouse L2 cells (a gift from Kathryn Holmes, University of Colorado, Denver, CO). Stock virus was propagated in L2 cells and concentrated by centrifuge at 100,000g for 2 hours, and the pellet was resuspended in DMEM with 2% FBS. Viral infectivity titrations were performed on L2 cells propagated in 96-well plates. Infectivity was recorded as the induction of cytopathic effect by serial 10-fold dilutions of the sample.

BALB/c mice were obtained from Harlan Sprague-Dawley (Indianapolis, IN). CD-1 mice were obtained from Charles River (Raleigh, NC). IFN- γ -deficient (IFN- γ gko) BALB/c mice were obtained from Jackson Laboratories (Bar Harbor, ME). All mice were between 10 and 14 weeks of age at the time of inoculation.

The mice were injected intravitreally with $10^{4.5}$ median tissue culture infective dose (TCID₅₀)/5 μ L JHM virus or with DMEM with 2% FBS as a mock control. The mice were anesthetized as described and killed by decapitation.¹ They were handled according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Mouse eyes were processed between 1 and 20 days after inoculation. The eyes were fixed in 10% formalin for hematoxylin and eosin staining or in optimal cutting temperature (OCT) compound for frozen sections and immunocytochemical staining.

Immunohistochemical Staining

Antibodies. Rabbit antiserum prepared against gradient-purified, NP40-disrupted MHV virions were kindly provided by Kathryn V. Holmes. Monoclonal antibody (mAb) specific for the MHV-JHM nucleocapsid protein N was generously donated by Julian Leibowitz (University of Texas, Houston, TX).

The following anti-mouse mAbs were used: for B cell identification, rat IgG2a reacting with CD45R (Pharmingen, San Diego, CA); for CD4⁺ T-cell identification, rat IgG2b reacting with L3/T4 (Sera-Laboratory, Belton, UK); for CD8⁺ T-cell identification, rat IgG2b reacting with Lyt-2 (Sera-Laboratory); for macrophage identification, rat IgG2b reacting with Mac-1 (CD11b/CD18), which identifies both macrophages and NK cells (Roche Diagnostics, Inc., Indianapolis, IN); for NK cell identification, mouse IgG2a reacting with 5E6 NK (Pharmingen); for MHC class I identification, rat IgG2a reacting with mouse H-2 monotypic antigen; and for MHC class II identification, rat IgG2b reacting with mouse Ia antigen (both from Roche Diagnostics, Inc.).

Immunoperoxidase Staining. Frozen sections were fixed in acetone for 5 minutes and immersed in Tris-buffered saline with 5% normal goat serum or horse serum for 10 minutes. Primary antibodies were applied. After incubation in a moist chamber at room temperature for 1 hour, the slides were washed in Tris-buffered saline, and the secondary antibody, biotin-conjugated goat anti-rabbit IgG (Organon Teknica Corp., West Chester, PA) or horse anti-mouse IgG (Vector Laboratories, Burlingame, CA), was layered onto the slides. After a 1-hour incubation in a moist chamber at room temperature, the slides were washed in Tris-buffered saline and overlaid for 45 minutes with avidin-biotin-peroxidase complex. The slides were washed again in Tris-buffered saline and developed in a 0.05% 3,3'-diaminobenzidine tetrahydrochloride-0.1% nickel sulfate-0.01% hydrogen peroxide solution. They were then counterstained with 1% methyl green, dehydrated, cleared, and mounted as in conventional processing.

Immunofluorescent Staining. Frozen sections were fixed in acetone, rehydrated in phosphate-buffered saline (PBS), and preincubated with normal goat serum, as described for immunoperoxidase

TABLE 1. Serum IFN- γ Levels in BALB/c Mice Infected with JHM Virus by the Intravitreal Route

Mice	Days after Inoculation		Total
	1-5	6-10	
Normal			
Positive/total	1/19	0/7	1/26
Percent positive	5.2	0	3.8
Mean (pg/mL)	92	0	67
JHM Infected			
Positive/total	4/26	5/10	9/36
Percent positive	15	50	25
Mean (pg/mL \pm SE)	203 \pm 112	715 \pm 282	346 \pm 117

staining. Primary antibodies were applied for 30 minutes in a moist chamber. Slides were washed three times in PBS followed by incubation with either fluorescein-conjugated goat anti-rat IgG or rhodamine-conjugated goat anti-rabbit IgG. They were again washed three times, mounted, and viewed by fluorescence microscope.

The following samples were evaluated in the immunofluorescence assay. Normal, mock-injected, or virus-infected eye sections were incubated with medium, irrelevant mAb, anti-MHC class I and II mAbs, virus-specific antibodies (rabbit), and normal rabbit sera. These tissues were then incubated with the second FITC-labeled anti-rat Ig antibody or the rhodamine labeled anti-rabbit antibody. The irrelevant mAbs were the same isotypes as the positive antibodies and were used at the same concentration as the sample being tested.

Reverse Transcriptase-Polymerase Chain Reaction

Each retinal RNA sample was dissected and pooled from eight mouse eyes. RNA was isolated with a commercially available protocol (RNA STAT-60; Tel-Test, Inc., Friendswood, TX). cDNA was synthesized from the same amount of RNA and was amplified with a kit (GeneAmp RT-PCR Kit; Perkin Elmer-Roche Molecular Systems, Inc., Branchburg, NJ). The specific primers were as follows: IFN- γ sense primer, 5'-TGC ATC TTG GCT TTG CAG CTC TTC CTC ATG GC-3', and antisense, 5'-TGG ACC TGT GGG TTG TTG ACC TCA AAC TTG GC-3'; and β -actin primers from Clontech Laboratories, Inc., Palo Alto, CA. PCR products were electrophoresed in 4% agarose gels. After denaturing and neutralizing, DNA was transferred to a nylon membrane, hybridized with specific internal digoxigenin (DIG)-labeled oligonucleotide or cDNA probes made from kits (Genius kits; Roche Diagnostics, Inc.).

RESULTS

Model System

In this report, as in our previous studies, the mock-injected and untreated mouse eyes did not reveal histopathological changes, 100% ocular infection of BALB/c mice with JHM virus resulted in retinal inflammation at postinoculation (PI) days 1 to 8 and retinal degeneration after PI day 10. In contrast, inoculation of JHM virus into CD-1 mice induced only the early inflammatory phase. At day 20, the CD-1 mouse retinas appeared normal.

IFN- γ Serum Levels

Initial studies were performed to determine whether IFN- γ was present in normal BALB/c mice and in BALB/c mice infected with JHM virus. Serum samples were collected between days 1 to 10 after intravitreal inoculation with JHM virus and were evaluated for IFN- γ by ELISA. As is seen in Table 1, IFN- γ was detected in only 1 (3.8%) of 26 serum samples from normal BALB/c mice. In contrast, IFN- γ was detected in 4 (15%) of 26 ECOR mice between PI days 1 and 5 and in 5 (50%) of 10 ECOR

mice between PI days 6 and 10. The highest levels of IFN- γ , 715 pg/mL, were detected in ECOR mice between PI days 6 and 10. These studies demonstrated that JHM infection in BALB/c mice was associated with an increase in both the incidence and levels of systemic IFN- γ .

IFN- γ Gene Expression within the Retina

We next wanted to determine whether IFN- γ was produced locally within the infected retina. Pooled retinal mRNAs from untreated, mock-injected, and virus-infected BALB/c mice were evaluated by RT-PCR for the expression of IFN- γ genes. Representative data from three separate experiments are shown in Figure 1. In virus-infected mice, IFN- γ mRNA was observed at PI days 4 and 8 and not at PI day 20. In normal and mock-injected animals, IFN- γ mRNA was not detected in retinal samples extracted on PI days 4, 8, and 20.

Correlation with CD4 and CD8 T-Cell Expression

IFN- γ is produced by T cells and NK cells.^{1,3} Moreover, IFN- γ can also play a central role in viral clearance by activating CTL to produce granzyme B, a molecule intimately involved in the effector functions of these cells. In the next series of experiments we evaluated cellular infiltration within the retina during ECOR (Table 2). The most prominent infiltrating cell was the macrophage. MAC-1 staining was detected in 50% (3/6) of the eyes examined at PI day 3 and in 100% of the eyes examined at PI days 6 (11/11) and 10 (9/9). At PI day 20, MAC-1 staining was detected in two of six eyes examined. The macrophage appeared to be the main cell type reacting with a staining pattern that was present throughout the neural retina with occasional staining of cells within the vitreous. Each section of the retinas with positive staining for MAC-1 contained 3 to 12 foci of reactivity. The second most prominent cell was the T cell. CD4⁺ T cells were present in 35% and 28% of the retinas at PI days 3 and 6, respectively. This was followed by a shift to CD8⁺ T cells, which were observed in 55% and 66% of the retinas at PI days 6 and 10, respectively. A low number of CD8⁺ T cells was still noted on day 20 in 50% of the mice. Each section of the retinas with positive staining for T cells contained one to five foci of reactivity. B cells were infrequently observed. In addition to mononuclear cell infiltration, we also infrequently observed neutrophils within the vitreous of in-

TABLE 2. Retinal Leukocyte Infiltration within the Retina during ECOR in BALB/c Mice

Animal	Days PI	Immunocytochemistry*		
		Mac	CD4	CD8
Normal		0	0	0
JHM virus-infected	3-4		35	0
	6-8	100	28	55
	10	100	0	66
	20	33	RT†	50

Eleven eyes were evaluated for normal eyes and 6 to 11 eyes were evaluated for JHM virus-infected eyes at each time point.

* Data are expressed as percent positive eyes.

† Reactive tissue. Tissue at this time point reacted with control reagents.

ected mice. NK cells were not seen within the infected retinas. Because IFN- γ is produced only by T cells and NK cells, these studies demonstrating that T cells were detected within the retina at a time when IFN- γ gene expression was observed support the concept that T cells are the source of IFN- γ .

Activation of MHC Classes I and II

The IFNs are potent inducers of MHC class I molecules, and IFN- γ also triggers the cellular expression of MHC class II molecules. When eyes from BALB/c mice infected with JHM virus were evaluated by immunocytochemical staining, reactivity for MHC class I and II molecules was observed on RPE cells, the inner retina, and the ciliary body epithelial cells (Figs. 2, 3). A comparison of MHC class I and II expression within the retina during ECOR is shown in Table 3. Within the retina, the RPE cells were the first type of cells to express both MHC class I and II molecules. This was noted at PI days 3 and 6. By PI day 10, cells within the neural retina also expressed both molecules. At PI day 20, MHC class I and II molecule expression was diminished, but in a pattern that differed between the two MHC molecules. MHC class I staining was decreased in both the number of cells stained and the intensity of staining. Both RPE cells and cells within the neural retina reacted positively for MHC class I. MHC class II staining was decreased in cells within the neural retina but was maintained on RPE cells. Outside the retina, the ciliary body epithelium, which is contiguous with the RPE, expressed MHC class I and II molecules in a manner similar to the staining pattern in RPE cells.

When eyes of untreated BALB/c mice or BALB/c mice injected with DMEM by the intravitreal route were evaluated by immunocytochemical staining, reactivity for MHC class I was not detected within the retina, RPE, or ciliary body epithelial cells (Table 3). Reactivity for MHC class II molecules was not detected in the retina or ciliary body epithelial cells but was observed on RPE cells in 2 of 11 eyes. This reactivity was patchy and weak.

To determine whether the same cells were expressing viral antigen and MHC molecules, we performed double-labeling experiments. The anti-MHC class I and II mAbs were incubated with FITC (green)-tagged goat anti-rat IgG, and the rabbit anti-JHM virus antibody was incubated with a rhodamine (red)-tagged goat anti-rabbit antibody. The slides were first viewed under a light microscope. The field was then examined with a rhodamine (red) filter and then with a fluorescein (green) filter. In double-exposure photography of the field, the cells reacting with both fluorescein and rhodamine-conjugated antibodies demonstrated a yellow-orange color. We found that RPE cells, ciliary body epithelial cells, and some Müller-like cells through-

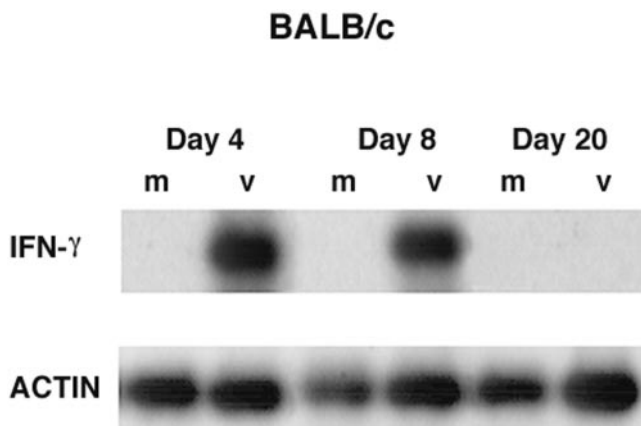
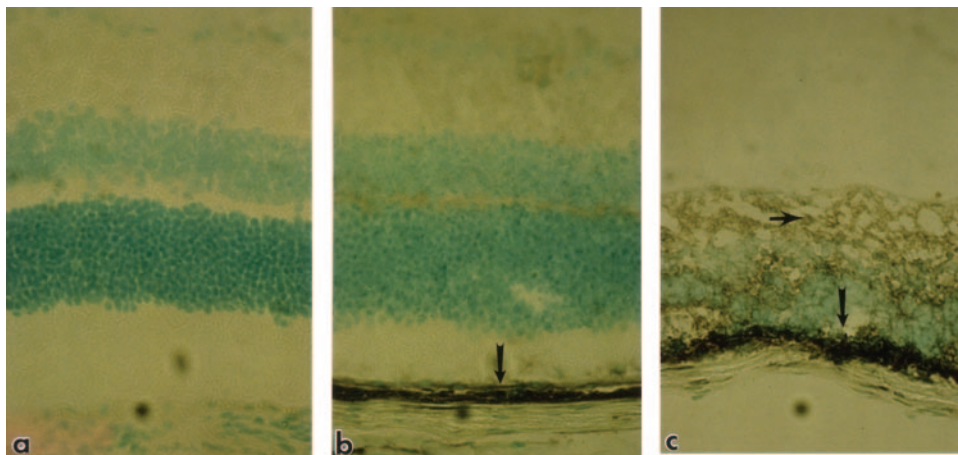


FIGURE 1. IFN- γ gene expression within the retina of BALB/c mice. Groups of BALB/c mice ($n = 8$) were inoculated by the intravitreal route with JHM virus (Virus) or DMEM (mock injected) or were untreated (control). At PI days 4, 8, and 20, retinas were harvested. Total RNA was isolated from the retina and analyzed for IFN- γ and β -actin mRNA by RT-PCR and Southern blot hybridization. Similar data were obtained in three separate experiments. m, mock injected; v, virus inoculated.

FIGURE 2. Immunocytochemical staining for MHC class I molecules within the retina of BALB/c mice. Frozen sections of (a) normal, (b) JHM virus-infected (PI day 6), and (c) JHM virus-infected (PI day 10) mouse eyes, were incubated with monoclonal antibody directed against MHC class I molecules. *Arrows:* positive (*black*) staining within the infected retina. No reactivity was noted when antibody was reacted with retinas from uninfected animals. Magnification, $\times 200$.



out the neural retina all react with the anti-virus antibody. By double-exposure photography it was determined that these virus-infected cells also expressed MHC class I and II molecules.

JHM Virus Infection in Mice Deficient in IFN- γ

To evaluate the role of IFN- γ in this ocular disease, JHM virus was inoculated into the vitreous of BALB/c mice (wild-type) and BALB/c mice deficient in IFN- γ secretion (IFN- γ gko mice). The wild-type mice responded to the virus infection by development of typical inflammatory disease and the secondary

retinal degenerative disease. Morbidity and mortality were compared between IFN- γ gko mice and wild-type mice infected with JHM virus. During the early phase of the disease only 2 of 14 wild-type mice were moribund or died. In contrast, 75% (12/16) of the IFN- γ gko mice became moribund or died at PI days 7 to 8, with clinical signs of encephalitis. The remaining mice were sick and were killed. These studies revealed increased mortality in IFN- γ gko mice, demonstrating an inability to control viral replication. These studies indicate that IFN- γ was a critical factor in limiting the pathologic consequence of the acute phase of this retinal disease.

FIGURE 3. Immunocytochemical staining for MHC class II molecules within the retina of BALB/c mice. Frozen sections of (a) normal, (b) JHM virus-infected (PI day 6), (c) JHM virus-infected (PI day 10), (d) JHM virus-infected (PI day 20) mouse eyes, were incubated with monoclonal antibody directed against MHC class I molecules. *Arrows:* positive (*black*) staining within the infected retina. No reactivity was noted when antibody was reacted with retinas from uninfected animals. Magnification, $\times 200$.

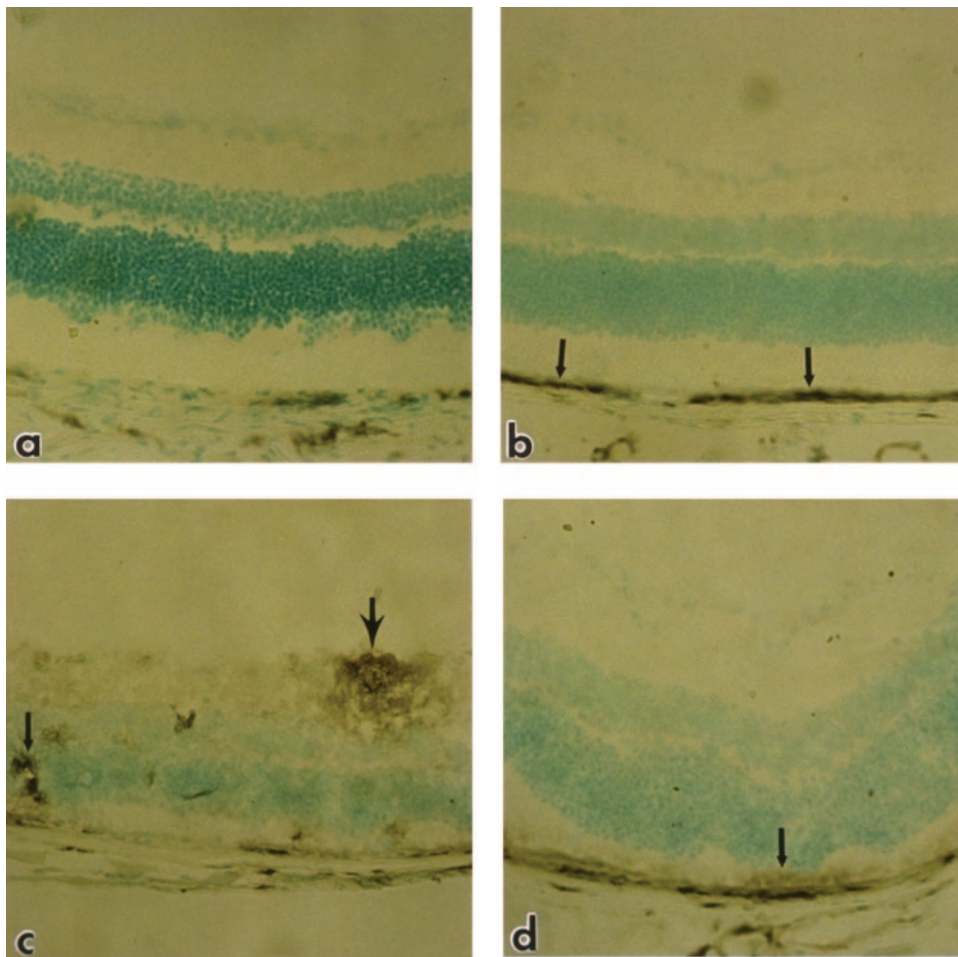


TABLE 3. Sequential Expression of MHC Class I and II Molecules with the Retina during ECOR in BALB/c Mice

MHC and Location	Normal Mice	JHM Virus-Infected Mice (Days PI)			
		3	6	10	20
Class I					
RPE	0/11	4/5	5/5	5/5	2/5
Retina	0/11	0/5	2/5	5/5	1/5
CBE	0/11	4/5	4/5	5/5	2/5
%Positive	0	80	100	100	40
Class II					
RPE	2/11	4/5	5/5	5/5	5/5
Retina	0/11	0/5	2/5	5/5	3/5
CBE	0/11	3/5	5/5	5/5	5/5
%Positive	18	80	100	100	100

Data are the number positive/total at each time point. CBE, ciliary body epithelium.

Comparison of BALB/c Mice and CD-1 Mice

When CD-1 mice were inoculated by the intravitreal route, acute disease developed that was similar to the inflammatory disease observed in BALB/c mice. However, CD-1 mice did not exhibit the late retinal degenerative phase of the disease. We therefore evaluated lymphoid trafficking, MHC class I and II expression, and IFN- γ gene expression in CD-1 mice and compared the findings with the data obtained in BALB/c mice. During the acute phase of the disease (days 3–10), lymphoid trafficking and MHC class I and II staining were similar in both mouse strains. Moreover, gene expression for IFN- γ in pooled retinas from CD-1 mice was positive at PI day 4 and 8 (Fig. 4). Again, this is similar to the pattern observed in BALB/c mice.

The disease pattern in the late phase was clearly different in the two mouse strains. BALB/c mice displayed a retinal degeneration and CD-1 mice showed a normal retinal architecture. Staining observed at PI day 20 in CD-1 mice was clearly different from that observed in BALB/c mice, in that CD-1 mice were negative for MHC class I and II reactivity, and CD8⁺ T cells were absent.

DISCUSSION

In the present study, the host responded to a coronavirus infection within the retina by recruiting monocytes and T cells into the infected retina and generating IFN- γ . When this retinal infection occurred in mice deficient in IFN- γ (IFN- γ gko mice) the virus was unchecked, and the infection led to death. These studies also demonstrated that IFN- γ was critically important in controlling a retinal virus infection. Several lines of evidence indicate that IFN- γ was acting at multiple levels. First, IFN- γ induced an antiviral state in uninfected cells resulting in decreased JHM virus replication (data not shown). Second, IFN- γ could augment the activity of T cells and CTLs. This may be facilitated by the upregulation of MHC class I expression on infected cells and by the induction of granzyme B in CTLs. We have shown in this report that MHC class I was induced within the infected retina and we have previously demonstrated the upregulation of granzyme B mRNA in the infected retina.²⁰ Third, IFN- γ activation of macrophages may result in production of additional cytokines, which could augment immune reactivity. Finally, IFN- γ can enhance immune reactivity through the induction of MHC class I and II molecules.²¹

IFN- γ is a major immunoregulatory protein that exerts its effects on a variety of cells and cellular functions. In virus infections, including coronavirus infections, this Th1 cytokine

has been shown to control virus replication. Infection of the CNS with the coronavirus, mouse hepatitis virus (MHV) JHM strain, is a rodent model of the human demyelinating disease multiple sclerosis.²² In this model, IFN- γ mRNA was detected at the time of viral clearance from neurons *in vivo*.^{23–25} Moreover, Parra et al.,⁴ demonstrated that both IFN- γ and virus-specific cytopathic activity inhibited JHM virus replication in the CNS by specifically acting on oligodendroglia cells. Recent studies have also indicated that virus-specific CD4⁺ T cells enter the CNS and produce IFN- γ during chronic demyelination.²⁶ In related studies, hepatitis induced by MHV was also shown to be less severe in mice treated with recombinant IFN- γ .²⁷

The importance of IFN- γ in both systemic and ocular viral infections have been explored in HSV-1 infections. Vollstedt et al.²⁸ clearly identified that IFN- γ and IL-12 play an essential role in controlling systemic and CNS infections with HSV-1. Bouley et al.⁵ evaluated herpes stromal keratitis in IFN- γ gko mice. Although corneal lesions resembling herpes simplex keratitis (HSK) occurred in IFN- γ gko mice, there were marked differences in virus replication. In IFN- γ deficient mice, virus persisted in the cornea longer, there were more severe skin lesions, and the mice were more susceptible to encephalitis. These parameters were a direct reflection of diminished viral clearance. In a separate study, Geiger et al.^{6,7} developed transgenic mice with ectopic expression of IFN- γ in photoreceptor cells (rho- γ transgenic mice).^{6,7} These mice showed photoreceptor loss, exhibited upregulation of MHC classes I and II, and showed increased infiltrate of macrophages into the retina. Moreover, these mice were protected from retinitis after intravitreal HSV injection. The authors suggested that IFN- γ provides protection through multiple mechanisms, such as activation of immune response, alteration of properties of the virus, and direct protection of neurons. The present study demonstrated for the first time the importance of IFN- γ in controlling JHM virus infection within the retina. The presence of IFN- γ in immune-associated ocular diseases has also been described. T cells infiltrating the retina were shown to express IFN- γ and IL-2 in patients with sympathetic ophthalmia and uveitis.²⁹ Animal models of autoimmune uveitis have also been used to show that IFN- γ gene expression augments disease activity.³⁰

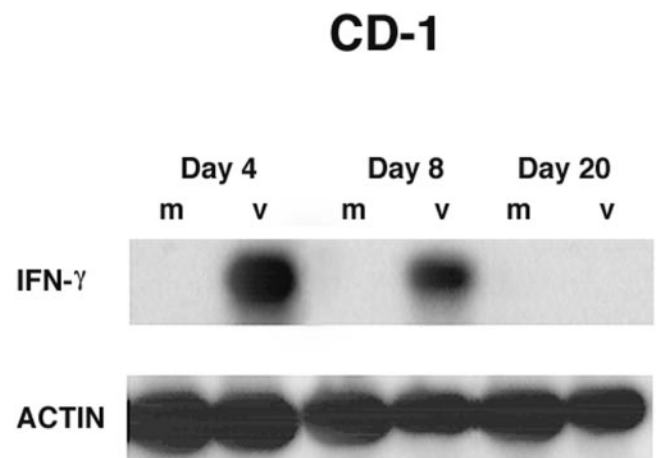


FIGURE 4. IFN- γ gene expression within the retina of CD-1 mice. Groups of CD-1 mice ($n = 8$) were inoculated through the intravitreal route with JHM virus (Virus) or DMEM (mock injected) or were untreated (control). At PI days 4, 8, and 20, retinas were harvested. Total RNA was isolated from the retina and analyzed for IFN- γ and β -actin mRNA by RT-PCR and Southern blot hybridization. Similar data were obtained in three separate experiments. m, mock injected; v, virus inoculated.

Thus, IFN- γ plays an important role in ocular inflammatory diseases, whether they are of infectious or noninfectious origin.

Virus invasion into the retina requires a rapid and measured response to downregulate virus replication and to establish viral clearance. The magnitude and timing of immune reactivity are critically important in controlling the virus in the presence of minimal immunopathology. We suggest that investigations using the animal model ECOR may shed light on some of the interactions among virus in the retina, the host cells and immune responses. ECOR was established by a virus that replicated and persisted within ocular cells in a nonlytic manner.^{17,18} In this infection, the host controlled the acute virus replication through a nonlytic process that preserved the infected cell. This is important in the retina, where cells are irreplaceable. Clearance of infectious virus from the retina was associated with the presence of CD4⁺ and CD8⁺ T cells without significant retinal damage in CD-1 mice. This suggests that clearance was a cytokine-mediated process. This process has been described for the clearance of Sindbis virus from the brain. Sindbis virus was cleared without neuronal damage, and IFN- γ was identified as a critical cytokine.¹² In the present study, IFN- γ played a critical role in limiting virus replication and spread within the retina. In CD-1 mice, this was sufficient to allow the retina to return to a normal architecture.

IFN- γ also played a critical role in limiting virus replication and spread within BALB/c mice. However, this nonlytic process of virus control has also been shown in a number of virus systems to be associated with progressive or chronic disease.^{31,32} Thus, the virus established a persistent infection in which the infected cell survived, setting up a pattern of progressive disease that may lead to immune-mediated damage. Earlier studies have shown that anti-retinal and anti-RPE cell antibodies are present in JHM virus-infected BALB/c mice.¹³ We hypothesize that this scenario of immune-mediated damage is operative in the JHM-infected BALB/c mice. The data presented herein show that CD8⁺ T cells were present within the retina beginning at PI day 6, in both mouse strains. However, at PI day 20, CD8⁺ T cells were present only in BALB/c mice. This coincides with a continued blood-retinal barrier breakdown in BALB/c mice.²⁰ The trafficking of CD8⁺ T cells within the retina at a time when retinal degenerative changes were noted suggests that these cells may have specificity not only for viral proteins but also for retinal determinants. Additional studies are needed to identify the generation of autoreactive CTLs in this model system.

It is hoped that development of this animal model of acquired retinopathy, ECOR, will define how specific host responses provide protection from retinal damage, whereas other host responses lead to progressive retinal disease. The results of the present study suggest that IFN- γ plays a critical role in protecting the host during a retinal infection.

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