WIDESPREAD AND SELECTIVE INDUCTION OF MAJOR HISTOCOMPATIBILITY COMPLEX–DETERMINED ANTIGENS IN VIVO BY γ INTERFERON

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The level of antigenicity of parenchymal cells was long assumed to remain fairly constant throughout the life of the organism (1), with the exception of a postnatal period of relative deficiency of histocompatibility antigens. It has become apparent recently (2) that exposure of certain lines of cultured cells to preparations rich in interferon (IFN) can increase the expression of surface antigens determined by genes of the major histocompatibility complex (MHC). Subsequent experiments with a number of types of cultured cells suggested that immune interferon, or γ interferon (IFN-γ), is the principal active substance in these preparations, in that purified IFN-γ (3) or recombinant IFN-γ (4) may be effective in increasing the expression of both class I and II MHC antigens on certain cells in vivo (5). Since both class I and II histocompatibility antigens are critical to the elicitation of T cell immune responses, it could prove to be of considerable importance if the cellular expression of MHC-determined antigens in vivo fluctuates in response to local humoral factors. Our interest was drawn to this possibility when we found that class II MHC antigens are promptly enhanced to high levels in transplanted mouse kidneys (6) and early in the course of autoimmune encephalomyelitis (7, 8). Subsequent observations have shown that this also pertains to some class I MHC antigens in the mouse kidney (unpublished observations) and other allografts such as the heart (9) and pancreas (10) in the rat.

The present report describes the alterations that we have seen in the expression of selected class I and II histocompatibility antigens in a variety of tissues of mice that have received a short course of daily, intraperitoneal injections of murine IFN-γ prepared by recombinant DNA techniques. This is the first report of a systematic evaluation of the effects of IFN-γ treatment on the expression of such antigens in vivo. Quantitative absorption of appropriate monoclonal antibodies has given us information concerning the magnitude of the increases in antigen expression.
expression that occurred under the conditions of these experiments. In addition, immunopathologic examination of tissues was used to determine the nature or the topography of the responsive cells and the new antigenic epitopes.

Materials and Methods

**Animals.** B10.BR (H-2<sup>b</sup>) and C57BL/6 (H-2<sup>b</sup>) male mice 6–10 wk of age were obtained from The Jackson Laboratory, Bar Harbor, ME.

**Antibodies.** An anti-H-2K<sup>k</sup> monoclonal antibody was used to evaluate both the amount and distribution of a class I histocompatibility antigen and an anti-I-A<sup>k</sup> monoclonal antibody to study a class II antigen (Becton, Dickinson & Co., Mountain View, CA). These were biotinylated in our laboratory according to a modification of a procedure outlined by Bethesda Research Laboratories, Gaithersburg, MD. A single lot of each antibody was used throughout the present study.

**IFN-γ.** Recombinant murine IFN-γ from E. coli, at a concentration of 1.75 mg/ml and containing 17 x 10<sup>6</sup> U/mg, was obtained through the generosity of Dr. Gene Burton, Genentech, Inc., South San Francisco, CA. It was stored at 4°C and diluted in phosphate-buffered saline (PBS) and 10% normal B10.BR serum immediately before use. IFN-γ was injected intraperitoneally daily for 6 or 9 d at a dose of 5 x 10<sup>4</sup> U/d. This dose was selected because it had been effective as an in vivo antiviral treatment (11). Higher doses, as described by Lindahl et al. (5), were assessed in pilot trials and proved to be toxic in our protocol. Additional animals were treated with IFN-γ for 9 d but not sacrificed until 1 or 2 wk later to assess the regression of the response.

**Absorption Assay.** The absorption assay used for the quantitative estimation of antigen expression in various tissues was a modification of that described by Wadgymar et al. (12). The antigenicity of sonicated tissues was determined by their capacity to reduce the complement-dependent cytotoxic titer of a standard monoclonal antibody against lymphocytes from donors of the appropriate genotype.

**Antigen Preparation.** Tissue samples were frozen, weighed, and homogenized in a glass mortar. RPMI 1640 medium was used at a ratio of 9.5 μl/mg of sample for both homogenization and washing. The suspension was sonicated for 1 min in a Branson Sonifier (Branson Sonic Power Co., Danbury, CT) at 45 W and 20 kHz frequency and then aliquoted and stored at -80°C until assayed.

**Antibody Dilution.** A suitable antibody dilution was selected on the basis of the following criteria: that it would kill >90% of B10.BR splenic adherent cells in a complement-dependent reaction, that absorption with a control C57BL/6 tissue preparation produced no detectable drop in cytotoxicity, and that absorption with a sample of the normal B10.BR tissue that was under study resulted in an easily detectable loss of cytotoxicity. A 1:40,000 dilution of anti-H-2K<sup>k</sup> antibody and a 1:20,000 dilution of anti-I-A<sup>k</sup> antibody met the above criteria for all tissue samples tested except brain. The amount of antibody contained in 1 μl of the final dilution of either of the antibodies was defined as a unit of antibody for the purposes of this study.

**Absorption.** 190 μl of a sonicated suspension containing 20 mg (wet weight) of tissue was distributed to eight tubes in doubling dilutions in RPMI 1640 containing 5% fetal calf serum. After dilution, each tube contained a volume of 95 μl, with the first tube containing the equivalent of 10 mg of tissue. 5 μl of a 1:1,000 or 1:2,000 dilution of the appropriate antibody was added to each tube to give the final dilutions of anti-H-2K<sup>k</sup> and anti-I-A<sup>k</sup> mentioned above. The tubes were incubated with constant shaking for 1 h at 4°C. They were centrifuged at 100,000 g for 15 min on an air-driven ultracentrifuge (Beckman Instruments, Inc., Palo Alto, CA), and the supernatants were then assayed for residual cytotoxicity.

**Cytotoxicity Assay.** Complement-dependent cytotoxicity of the antibody preparations was determined using dispersed B10.BR splenic adherent cells as described previously (13). Final absorption activity was expressed as the number of units of antibody absorbed by the sonicate prepared from 1 mg of tissue and converted into multiples of the activity of the mean value for normal kidney.
Light Microscopy. Tissue from various organs was snap frozen and processed as described (7, 8). 4-μm cryostat sections were fixed in acetone and stained with the avidin-biotin-peroxidase complex technique (7). Tissues from two to three animals were examined for each time point, including samples from both normal B10.BR mice and the nonreactive strain, C57BL/6.

Dendritic Cell Counts. I-Ak-positive interstitial cells were counted at a magnification of 312 using an ocular micrometer grid. The cells visible in 0.3 mm² randomly selected fields were counted on each of 10 slides prepared from each tissue specimen subjected to evaluation. Results were averaged and were expressed as the number of I-Ak-positive cells/mm².

Electron Microscopy. Slices of tissue 1 mm in thickness were fixed immediately in Nakane's fixative (2% paraformaldehyde, 0.075 M lysine, and 0.01 M sodium periodate in 0.0375 M phosphate, pH 7.4) (14) for 50 min at room temperature and rinsed in PBS. Free aldehyde groups were quenched with 0.05 M glycine in PBS for 45 min at room temperature and the tissue stored in PBS at 4°C. Sections of fixed tissue 100 μm thick were cut on a Vibratome (Lancer Co., St. Louis, MO) and stained by the avidin-biotin-peroxidase complex method. After incubation for 1 h in normal rabbit serum, further incubations for 30 min each in avidin (100 μg/ml in PBS) and biotin (10 μg/ml in PBS) were carried out. All incubations were followed by three 10-min washes with PBS. The sections were then left overnight at room temperature in 100 μl of biotinylated antibody at the following dilutions: anti-I-Ak at 1:25 or anti-H-2Kk at 1:10. The following day, after washing in PBS, endogenous peroxidase activity was blocked with 0.3%

Results

Absorption Assay

Class I antigen (H-2Kk). The results of absorption studies of selected B10.BR tissues in the resting state (day 0) and on days 6 and 9 after treatment with IFN-γ are presented as the relative antigenic potency of various tissues compared with the antigenic activity of the normal kidney (Fig. 1). In normal mice the liver and spleen had 14–19 times the antigen concentration of the kidney. The lung, lymph node, and thymus were six to eight times richer than the kidney as antigen sources, while the heart and adrenals were twice as rich. Skeletal muscle, pancreas, and small intestine were only about half as active as kidney.

Treatment with IFN-γ resulted in a definite increase in the expression of H-2Kk antigenic epitopes in all tissues studied except lymph node and perhaps thymus (only one specimen tested). Spleen, liver, and lung, which had high antigenic activity in the resting state, showed a less pronounced increase in activity after treatment with IFN-γ. Heart, kidney, and adrenals, on the other hand, showed an increase in antigenicity to 4–8 times their previous values, while pancreas and small intestine responded with the largest increase seen, 13- and 17-fold levels of H-2Kk antigen expression, respectively. Skeletal muscle showed very low levels of activity both before and after stimulation.

Class II antigen (I-Ak). In the resting state, spleen, lymph node, and thymus displayed considerably more antigen than any other organs (Fig. 2). Lung showed
activity five times higher than the normal kidney, but was still less active than any of the lymphoid organs. Heart, kidney, liver, and adrenals manifested similar activity, while skeletal muscle, pancreas, and small intestine were the least active.

After treatment with IFN-γ, lymphoid organs did not show an increase in their ability to absorb anti-I-A<sup>k</sup> antibody. Heart, kidney, pancreas, lung, liver, adrenal, and small intestine, however, responded to the lymphokine with a marked increase, reaching levels of 6–10 times that of normal kidney tissue. Skeletal muscle showed low activity in the resting state, and remained low after treatment. Class I and II antigenic activity of the brain was present in amounts
below the sensitivity of our method to detect it both before and after stimulation with IFN-γ.

Morphologic Distribution of H-2^k and I-A^k Antigens in Normal Mice

Hematopoietic/lymphoid system. In normal mice the class I antigen (H-2^k) was detected primarily in lymphoid organs, namely the thymus, lymph nodes, and spleen. Almost all reactivity in the thymus was in the medulla, where reactive cells often had a broad and scalloped appearance, typical of thymic epithelial cells. In lymph nodes, scattered islands of more strongly positive lymphocytes were present in the medulla and primary follicles. All lymphoid areas in the spleen were positive, particularly the eccentric B cell regions.

Lymphoid tissues had the most abundant basal level of the class II antigen (I-
A\(^1\)). In the thymic cortex, epithelial cells were positive, with a characteristic scalloped pattern. In the medulla many larger cells with abundant cytoplasm were intensely stained. The lymphocytes in the B cell–rich eccentric nodules of the spleen and primary follicles of lymph nodes were uniformly positive (Fig. 3), while lymphocytes in the T cell–rich splenic periarteriolar sheaths and the

**Figure 3.** Bone marrow (a, b) and spleen (c, d) sections stained for I-A\(^1\). The number of I-A\(^1\)-positive cells increased dramatically in the bone marrow after 6 d of treatment with IFN-\(\gamma\) (b), compared with normal marrow (a). The lymphocytes in the B cell nodules in the normal spleen stain well for I-A\(^1\) (c) and showed little change after 6 d of IFN-\(\gamma\) (d). In contrast, there was a marked decrease in the intensely staining dendritic cells (D). (a, b) \(\times 313\); (c, d) \(\times 125\).
paracortex of nodes were negative. Strongly positive dendritic cells were present in T and B cell regions of the spleen and nodes (Fig. 3). ~5% of the nucleated cells in the bone marrow were positive for I-A<sup>k</sup>, but none had detectable H-2K<sup>k</sup>.

**Nonlymphoid organs.** Aside from occasional class I-positive cells believed to be in vascular spaces (e.g., the sinusoids of the spleen and alveolar walls of the lung), the only class I antigen detectable with our standard immunohistochemical technique in the nonlymphoid tissues was in the base of the epididymal epithelium and the lamina propria of the villi of the small intestine. Little or no class I antigen was detectable on parenchymal, dendritic, or vascular cells of the kidney, liver, pancreas, salivary glands, heart, skeletal muscle, lung, adrenal glands, thyroid, testis, or brain. When the incubation period was extended to 18 h at 4°C and the antibody concentration increased, both to the point of nonspecific staining in control C57BL/6 tissue, patchy staining was evident in the liver of normal B10.BR mice.

Class II antigen, in contrast, was regularly found in the connective tissue of most organs on large, elongated, irregular cells, described by others as dendritic cells (18). Organs with readily discernible dendritic cells included the heart (Fig. 4), kidney (restricted to the cortex, especially around vessels at the cortico-medullary junction), liver (in portal tracts, not in sinusoids), pancreas (0-4 dendritic cells per islet cross section), salivary glands, small intestine (lamina propria), heart, skeletal muscle, lung (perivascular and subpleural), adrenal gland (especially the zona glomerulosa and medulla), thyroid, and epididymis. No class II antigen was detectable in the brain, testis, renal medulla, or on parenchymal cells in nonlymphoid organs.

**Distribution of H-2K<sup>k</sup> and I-A<sup>k</sup> Antigen after IFN-γ Treatment**

Treatment with IFN-γ caused a widespread, but selective, increase of class I and II antigens in most tissues. This effect was well established after 6 d of treatment and sometimes increased further by 9 d.

**Lymphatic/hematopoietic system.** The bone marrow showed a marked increase of both class I and II antigen, so that 20–30% of the nucleated hematopoietic cells became positive (Fig. 3). Lymphocytes in the spleen showed no obvious increase in class II antigen, although the lymphocytes had increased class I antigen in the marginal sinuses of the spleen, the cortex of lymph nodes, and focally in the outer thymic cortex. In addition, class II antigen–positive thymic epithelial cells were enlarged and more numerous in the cortex. In contrast to all nonlymphoid organs (see below), the intensely stained, elongated dendritic cells in the lymph nodes and spleen were transiently depleted after 6 d of IFN-γ treatment (Fig. 3).

**Nonlymphoid organs.** After 6 d of IFN-γ treatment, most tissues showed increased class I antigen expression on endothelial cells and more numerous dendritic cells bearing the class II antigen. Parenchymal cells showed a remarkably selective response to IFN-γ that varied according to the organ, the cell type within the organ, and even to cell membrane polarity.

(a) Vascular endothelium. This showed differential sensitivity to IFN-γ. The capillaries and venules were more responsive than arteries and veins in all organs. An increase in endothelial expression of the class I antigen was detected in the
FIGURE 4. Heart sections from normal (a, c) and 9 d IFN-γ-treated mice (b, d, e) stained for H-2K^k (a, b) and I-A^k (c, d, e). Arteries normally had little H-2K^k (a), but a marked increase in arterial endothelial H-2K^k occurred after IFN-γ (b). The number of interstitial I-A^k-positive cells increased several-fold with IFN-γ (c vs. d). Electron microscopy revealed that these I-A^k-positive cells were dendritic cells (D); adjacent capillary endothelium was negative (C). (a, b) × 313; (c, d) × 256; (e) × 8,500.
Liver sections stained for H-2K. The normal liver showed weak, diffuse staining of hepatocytes (a). After 6 d of IFN-γ, the sinusoids became strongly and uniformly positive (b). Electron microscopy showed that this sinusoidal staining was largely due to H-2K expression on the microvillar surface of hepatocytes (arrowheads) (c). Kupffer and endothelial cells also displayed H-2K staining. The hepatocyte membrane staining did not extend beyond the tight junctions, and the bile canaliculi were negative (insert). (a, b) × 125; (c) × 7,100; (insert) × 9,400.
heart (Fig. 4), diffusely along the sinusoids in the liver (Fig. 5), and in the spleen, vasa rectae of the kidney medulla, pancreas, small intestine, epididymis, testis, and brain (rare). Only a few small arteries in the pancreas, heart, spleen, and kidney expressed class I antigen. Positive vessels were not evident in the other organs examined (lung, salivary glands, adrenals, thyroid, muscle, and bone marrow). No artery or vein expressed detectable class II antigen. In contrast, mesothelial cells of the peritoneal cavity and pericardium did express class II antigens, and those of the peritoneum also expressed class I antigen.

(b) Dendritic cells. These were increased in all nonlymphoid organs, including the heart (Fig. 4), liver (Fig. 6) (portal areas), salivary glands, small intestine, kidney (Fig. 7), thyroid, adrenal medulla, testis, epididymis, brain (choroid plexus), skeletal muscle, and pancreas (islets and acini) (Fig. 8). These organs all normally contained class II antigen–positive dendritic cells except the medulla of the kidney and the testis, which had no class II antigen–positive interstitial cells until after IFN-γ treatment. Increased staining of macrophages for class II antigen was evident in the subcapsular sinuses of lymph nodes, pulmonary alveoli, as well as in the hepatic Kupffer cells (Fig. 6).

Immunoelectron microscopy permitted definitive identification of capillary endothelial cells and dendritic cells. The interstitial class II antigen in the kidney and heart (Fig. 5) proved to be restricted solely to elongated cells containing some peroxidase-positive vacuoles that clearly belonged to the dendritic cell class. No class II antigen was detected on endothelial cells after IFN-γ treatment in any tissue examined by electron microscopy (kidney, heart, lung, and liver). In the liver, Kupffer cells expressed both class I and II antigens, but endothelial cells expressed only class I (Figs. 4, 5). No dendritic cells were detected in the lung.

(c) Parenchymal cells. Cells of most organs showed increased expression of class I and/or class II antigens with IFN-γ treatment. However, within organs there was a notable heterogeneity. In the kidney, proximal tubules became positive for both class I and II antigens, but distal tubules and collecting ducts had substantially less class I and no detectable class II antigens (Fig. 7). In the pancreas, the ductal epithelium developed intense staining for class I and class II antigens, but the acinar cells showed no class I antigen and only focal patches of class II antigen (Fig. 9). In the salivary glands, the ducts and serous acini stained intensely for class I antigen, while the mucinous acini were negative. The basal epithelial staining in the small intestine was confined to the crypts, while luminal surface reactivity was seen throughout the villi. In the adrenal, the zona reticularis and zona fasciculata (glucocorticoid cells) expressed only class I antigen, while the zona glomerulosa (mineralocorticoid cells) expressed only class II antigen. In islets, the class I antigen was seen only on beta cells identified by electron microscopy (Fig. 9).

Thus, many epithelial cells responded to IFN-γ by increased expression of class I and II antigens (proximal tubules, small intestine, pancreatic ducts, hepatocytes, epididymis). Some parenchymal cells displayed only class I antigen (salivary gland ducts, beta cells, adrenal glucocorticoid cells, and distal tubules), and others expressed only the class II antigen (pancreatic acinar cells, type II pneumocytes, and adrenal mineralocorticoid cells). No induction of either class
FIGURE 6. Liver sections stained for I-A^k. The normal liver had scattered I-A^k-positive dendritic cells, largely around portal tracts, while Kupffer cells were negative (a). After 9 d of IFN-γ, discrete intensely positive cells were found in sinusoids, while hepatocytes remained negative (b). Electron microscopy showed that the intrasinusoidal cells were Kupffer cells with plasma membrane I-A^k (c). (a, b) × 160; (c) × 8,500.
FIGURE 7. Kidney stained for H-2Kk (a, b) and I-Ak (c, d, e). The normal kidney had little or no detectable H-2Kk (a), but its expression was markedly enhanced after 9 d of IFN-γ (b). Proximal tubules, Bowman’s capsule, vascular endothelium (arrowhead), and cells in glomerular tufts became positive (arrow). The staining in proximal tubules was intense and basal, while adjacent distal tubules were negative (*). I-Ak was restricted to occasional dendritic cells in the normal kidney cortex (c). After 9 d of IFN-γ a marked increase in I-Ak occurred in proximal tubules and contiguous Bowman’s capsule; distal tubules (*), collecting ducts, glomerular tufts, and vessels remained negative (d). A few tubules had adjacent positive and negative cells (arrowhead). Electron microscopy showed that the basal staining of proximal tubules was due to I-Ak reactivity along the basolateral plasma membrane. Reaction product did not extend beyond the intercellular tight junction (arrowhead) and the brush border (top) was negative (e). (a, b) × 313; (c) × 200; (d) × 125; (e) × 8,500.
FIGURE 8. Pancreas sections stained for I-A$^b$ (a, b, d). The normal pancreas had scattered I-A$^b$-positive cells in the islets (l) and around ducts. After 9 d of IFN-γ, the number of intraislet (l) and interacinar I-A$^b$-positive cells increased and ductal epithelium became positive (arrowheads) (b). Adjacent section stained with toluidine blue (c). The intraislet I-A$^b$-positive cells were dendritic cells intercalated between β cells (d). The nearby capillary endothelium were negative (c). (a) × 313; (b, c) × 125; (d) × 6,250.
Figure 9. Pancreas sections stained for H-2K^k (a, b, d). The normal pancreas (a) had little detectable H-2K^k in islets (I) or acini, but after 9 d of IFN-γ (b), many cells in the islets (I) became positive, as well as the ductal epithelium (arrowheads) and vessels. An adjacent section stained with toluidine blue (c). Electron microscopy (d) shows that the positive cells in the islets were β cells (BC). (a) × 200; (b, c) × 125; (d) × 7,700.

I or II antigens was detectable immunohistochemically in striated muscle (heart and skeletal), seminiferous tubules, bile ducts, mucinous acini of salivary gland, or collecting tubules of the kidney.

In general, epithelia expressed higher antigen density on the side facing the blood, i.e., the basal surface. This was clearly evident for both class I and II antigens in the proximal tubules of the kidney (Fig. 7), and in small intestine, salivary gland, pancreatic ducts, epididymis, and thyroid. Similarly, hepatocytes showed increased class I antigen on their sinusoidal surfaces.
Electron microscopy confirmed that the expression of both class II and I antigens was restricted to the surfaces of parenchymal cells that faced blood vessels. In the kidney, the exterior aspect of the infolded basolateral cell membrane of proximal tubules became positive for both the class II and I antigens (Fig. 7). However, reaction product did not penetrate beyond the level of the tight junctions, and the luminal brush border was negative. Similarly, in the small intestine, the expression of class I antigen was confined to the basolateral membranes of the crypt epithelium. Hepatocytes were positive on their microvillar sinusoidal surfaces for class I, but not class II, antigens (a few patches of hepatocytes were positive for class II antigen by light microscopy). No staining for either was evident on the plasma membrane forming the bile canaliculi (Fig. 5).

**Class I and II Antigen Expression and Numbers of Dendritic Cells After Withdrawal of IFN-γ**

In the heart, antigens of both classes were present in larger than normal quantities 1 wk after withdrawal of the exogenous lymphokine (Fig. 10). By day 23, class I antigen activity had returned to normal while the class II antigen activity remained somewhat elevated. As judged by immunohistochemical staining, the expression of class I antigen returned to near baseline 7 d after withdrawal of IFN-γ. Dendritic cells remained slightly but definitely increased
in the kidney and heart, and Kupffer cells were more prominent in the liver even 2 wk after IFN-γ.

_Dendritic cell counts._ The normal heart and kidney had a similar density of dendritic cells, 12 and 10 cells/mm² (Fig. 10). After 3 d of treatment with IFN-γ, the number of cells tripled in the heart and more than doubled in the kidney, and continued to show an increase in the heart on days 6 and 9. Intense staining of tubular cells in the kidney obscured dendritic cells on day 6 and 9. One week after discontinuation of IFN-γ, little class I and class II antigen remained on tubular epithelium, but large numbers of dendritic cells could be identified (63 cells/mm² on day 16 and 39 cells/mm² on day 23).

**Discussion**

These experiments show that the level of expression of histocompatibility antigens can undergo marked and rapid changes throughout the body following the administration of IFN-γ. Although induction of both class I and class II antigens by this treatment is widespread, it also is remarkably selective within a given organ.

One notable feature of the tissue distribution was the restriction to certain parenchymal cells. The explanation for this may lie in part in the access of IFN-γ to cells. We presume that the expression of the MHC antigens is a local response to IFN-γ, not requiring intermediate agents, since in vitro IFN-γ is a sufficient stimulus for induction of class I and II antigens on many cell types, such as macrophages and endothelial, epithelial, and glial cells. Since the IFN-γ is delivered intraperitoneally and must then be absorbed and distributed throughout the body, some of the differences in antigen induction on the part of various cells might be due to uneven permeation of IFN-γ into different tissue compartments, even though its molecular size is small (15,894 daltons; the protein based on coded DNA sequence) (19) and the period of treatment of animals was as long as 9 d. Selective exposure to IFN-γ does not explain the differential response of proximal and distal tubules of the kidney, which are adjacent and share the same fenestrated capillary bed, or the parietal epithelium (Bowman’s capsule) and visceral epithelium (podocyte) of the glomerulus, which shared the same exposure to plasma filtrate. Clearly, cellular differentiation is one of the determinants of responsiveness to MHC antigen induction. This difference was seen in many organs, such as the pancreas (acini vs. ducts), salivary glands (serous vs. mucinous acini), and adrenals (zona glomerulosa vs. fasciculata). It is known from in vitro studies (20) that certain types of cells, such as T lymphocytes, do not express increased amounts of antigen when exposed to IFN-γ under conditions that result in new antigen expression by other cells. Considerable variation in the magnitude of increased antigen expression has also been found among various cultured lines of normal and tumor cells (21).

We were unable to detect class I antigens in most normal parenchymal organs using the standard protocol for staining. This does not exclude their presence, of course, but means that the level of expression was substantially lower than in normal lymphoid cells or parenchymal cells after IFN-γ. Indeed, when the staining was pushed to the limit of nonspecific binding, faint H-2Kk staining was visible in the hepatic sinusoids. The antigens are more readily detectable in the
liver by absorption techniques, presumably because they are diffusely distributed rather than concentrated on a few cells, as is the case of I-A\(k\) on dendritic cells.

The specificity of the response extended to whether class I, class II, or both antigens were expressed. For example, vascular endothelium often displayed enhanced class I antigens, but never showed increased class II. This result differs from that reported by de Waal et al. (22), who reported that skin graft vessels increased their class II antigen expression in the course of skin graft rejection. This may be a special case in that the endothelium is a direct target of the graft rejection process. Alternatively, the antigen they detected, which was not identified other than as an I region product, may be expressed more readily by endothelial cells than I-A products. Human endothelial cells normally display DR but not DQ (unpublished observations). Furthermore, certain human tumor lines respond to IFN-\(\gamma\) by DR and not DQ induction (23).

Perhaps the most striking aspect of the specificity of the response was the restriction of the antigen induction to certain portions of the cell membrane. This was clearly documented in the proximal tubules of the kidney, the hepatocytes, and the small bowel epithelium. In all cases the I-A\(k\) or H-2K\(k\) antigens were on the membrane facing the vascular space. A similar observation was made by Parr and Kirby (24) in 1979 on enzymatically dissociated cells from the small bowel, endometrium, and trachea of the mouse, using antiserum to H-2\(b\) determinants not otherwise specified. While somewhat artificial, their finding helps exclude the possibility that the distribution we found in situ is an artifact of penetration.

Membrane asymmetry of epithelia is well recognized, particularly for receptors and enzymes (25). Maintenance of asymmetry requires two minimal properties: (a) that the membrane components be selectively transported and inserted in one region of the membrane, and (b) that diffusion in the plane of the membrane is restricted between the two regions. The site of the diffusion barrier is the tight junction through which proteins bound to the membrane, such as lectins, cannot pass (26). This fits well with our in vivo observations of the ultrastructural distribution of the I-A\(k\) and H-2K\(k\) molecules, which end abruptly at the cell junctions. What is not yet explained is the mechanism by which the cell directs these components to one region of the cell or another. One question that arises is why the cell expresses MHC antigens on select membrane regions. Since exudate cells enter from the vascular side of the epithelium, this face is the one encountered by the emigrating T cell.

Three hypotheses can be advanced to account for the prominent increase in dendritic cells: (a) induction of I-A\(k\) on dendritic cells already present that normally lack this antigen, (b) local proliferation of dendritic cells, or (c) recruitment of dendritic cell precursors from the blood. Others (27) have given evidence for Ia-negative dendritic cells (identified by the W2/25 antigen). Indeed, these cells showed a predilection for the renal medulla, one site where IFN-\(\gamma\) caused I-A\(k\)-positive dendritic cells to appear de novo. We have no evidence for or against local proliferation, although mitotic figures were not seen, and the increase was large after only 3 d of treatment. Recruitment of precursors must be considered a likely possibility in view of the depletion of I-A\(k\)-positive dendritic
cells in the spleen and lymph nodes, and the increased number of I-A^k cells in the bone marrow.

Because cytotoxic and helper T cells only respond to antigen in conjunction with class I and II antigens, respectively, we believe that an important activity of IFN-\(\gamma\), as a normal part of the immune defense system, may be to increase the T cell clearance of target cells infected by viruses and to increase the immunogenicity of microbes by increasing the expression of MHC antigens at the site of invasion. Such increased immunogenicity of parenchymal cells could also be detrimental if it were to promote autoimmune responses (7).

It is not possible as yet to relate these findings to "normal" immunological events. Our findings with various doses of IFN-\(\gamma\) suggest that the phenomenon of antigen induction probably occurs with a wide range of levels of exposure to that lymphokine. Induction of large amounts of both class I and II antigens has been observed in vivo by Wadgymar et al. (12) in the kidneys of mice undergoing systemic graft vs. host reactions, in transplanted mouse kidneys in our own previous studies (6), and in transplanted rat hearts by Milton and Fabre (9). Similar reports of antigen induction have appeared in transplanted human kidneys (28, 29).

Thus, our findings make it entirely plausible to suspect that the level of expression of histocompatibility antigens varies widely from time to time on many types of cells during the ordinary vicissitudes of life. It is well known that encounters with various viruses or with many bacterial organisms, notably those in the Brucella or Listeria class among many others (30), will stimulate interferon production. Thus, changing levels of antigen expression, not only on macrophages and certain other migratory cells but also on many cells in fixed tissues, are likely to be common accompaniments of many forms of infection and perhaps of other pathological processes in which immune responses are involved. It is also of interest that antigen expression, at least in heart and kidney, diminishes quite rapidly after the cessation of IFN-\(\gamma\) treatment. This suggests the possibility of both rapid upward and downward regulation in antigen expression.

**Summary**

\(\gamma\) Interferon (IFN-\(\gamma\)) caused remarkable increases in class I (H-2K^k) and class II (I-A^k) antigens throughout the body by 6–9 d. Heart, kidney, and adrenals showed increases of 4–8 times their previous levels of class I antigen content, while the pancreas and small intestine increased 13–17-fold. Lesser increases were found in spleen, liver, and lung, which showed higher resting antigenic potency. Increases of class II antigenicity of 6–10-fold were found in heart, kidney, pancreas, lung, liver, adrenal, and small intestine, with lesser increases in thymus and spleen, and none in lymph node.

Topographical analysis revealed that IFN-\(\gamma\) induced class I and II antigens on most tissues in a highly selective fashion. For example, the renal proximal tubules expressed large amounts of both class I and II antigens, whereas the distal tubules and collecting ducts did not. In some epithelial cells class I and II determinants were induced only on the basal aspects of the cell membrane.

IFN-\(\gamma\) caused a remarkable increase in class II-positive dendritic cells in the liver, pancreas, salivary glands, and thyroid. Whether these cells were of local or
systemic origin is uncertain, but the finding of a simultaneous depletion of dendritic cells from lymph nodes and spleen raises the possibility that they may have been derived, at least in part, from these sites. The dynamic and selective induction of class I and II antigen expression by IFN-γ is likely to be important in regulation of the immune response in tissues.

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