ANTAGONISTIC EFFECTS OF γ INTERFERON AND STEROIDS ON TISSUE ANTIGENICITY

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Human kidney displays the class II MHC antigens on tissue dendritic cells (DC)[1] and on vascular endothelial cells (1–4). In addition, variable amounts of class II antigens are present in the cytoplasm of proximal tubular cells (4). Rat kidney is different. In addition to being present on the DC, large amounts of intracellular class II antigen are seen inside proximal tubular cells; on the other hand, very few kidney vascular endothelial cells express class II antigens, and in those that do, the level of expression is low (5–8). Human and rat heart and liver are similar. Thus in both organs in both species the level of class II expression on endothelial cells is low and the major class II antigen–expressing cells are the DC. In addition, in the liver, (some of) the Kupffer cells (tissue macrophages) are strongly class II–positive. The remaining parenchymal structures in the above three organs do not express detectable amounts of class II (9–11).

Herein, we show that the level of class II antigen expression in rat organs is not stable, but may be modulated by exogenous administration of drugs. rIFN-γ and steroids have antagonistic effects on class II antigen presentation in two separate antigenic compartments: on the presentation of the antigen by the graft parenchymal (endothelial) cells, and on the number of highly antigenic DC in tissue.

Materials and Methods

Rats. The nucleus of the inbred DA (AgB-4; RT1ᵃ) strain was obtained from Professor J. Gowans, Dunn School of Pathology, Oxford, United Kingdom. Male rats weighing ∼200 g were used for the experiments.

Reagents. Mouse IgG mAb against rat class II MHC antigens, MAS 029 (monomorphic, reactive to all class II molecules) was purchased from Sera-Lab, Ltd., Crawley Down, Sussex, United Kingdom. FITC-coupled goat anti–mouse IgG and tetramethylrhodamine isothiocyanate (TRITC)-coupled goat anti–rabbit IgG were obtained from Cappel Laboratories, Cochranville, PA. Rabbit antiserum to factor VIII–related antigen(s) (FVIII-RAg) was obtained from Dako Immunoglobulins a/s, Copenhagen, Denmark.

rIFN-γ was produced in the laboratory of H. Schellekens as follows. The protein was

[1] *Abbreviations used in this paper:* DC, dendritic cell; FVIII-RAg, factor VIII–related antigens; MP, methylprednisolone; TRITC, tetramethyl rhodamine isothiocyanate.

prepared from cultures of a transformed Chinese hamster ovary (CHO) cell line carrying the gene encoding rat IFN-γ. The methods of production and partial purification have been described previously (12). The preparation had sp act of ~2-4 × 10^7 U/mg protein. Methylprednisolone (MP) (Solu-Medrol) was purchased from Upjohn, Kalamazoo, MI, and dissolved in PBS before administration.

Double Indirect Immunofluorescence.   Tissues were embedded in Tissue Tek II (Miles Laboratories Inc., Naperville, IL), frozen in liquid nitrogen and cut using a Reichert-Jung microtome (Frigocut Model 2.700; R. Jung Gmbh, Nussloch, Federal Republic of Germany) at −15°C. For double indirect immunofluorescence, 5-μm frozen sections were dried in air, fixed for 10 min in acetone at room temperature, and double stained as follows: sections were exposed for 30 min at room temperature in a humidified atmosphere to a mixture of MAS029 antibody and rabbit anti-FVIII-RAg, they were then washed three times in PBS before being exposed to a mixture of FITC-coupled goat anti–mouse Ig and TRITC-coupled goat anti–rabbit Ig. After three washes in PBS, the slides were air dried and mounted in Aquamount (BDH Chemicals, Ltd., Poole, United Kingdom). Upon examination of the specimens in a Zeiss Universal Microscope equipped with an epi-illuminator IIIRS and filters for FITC and TRITC fluorescence, the strongly class II–positive DC were easily distinguished from the class II–positive vascular endothelial cells also expressing the FVIII-RAg, and vice versa.

Cells were counted at ×400 using a mesh (area, 55,225 μm^2). 10–20 fields were included in the analysis from each area. In the liver, counting started either from the portal vein or from the central vein. In this study the liver portal area was defined as being in the immediate vicinity of the portal vein, hepatic artery, and bile duct, to the extent of periportal connective tissue; the remaining part of the liver was called the hepatocellular or central vein area and is referred to herein as the central vein area.

As no unequivocally accepted marker antibody for rat DC exists at present, DC were defined as highly class II–reactive, nonphagocytic, nonparenchymal cells with dendritic morphology and not expressing FVIII-RAg (9, 13). To visualize phagocytic cells, the recipient rats were injected with 20% India ink in 0.5 ml of PBS 2 h before extermination (9). Phagocytic cells were easily identified by the ingested ink particles. The vascular (capillary) endothelial cells, which overlapped with DC especially in the renal intertubular space (7, 9, 13), were recognized by their expression of the FVIII-RAg (13).

Results

3 d after a single i.p. injection of rIFN-γ into a DA rat, the number of Ia⁺ FVIII-RAg⁻ DC increased in heart tissue 3.6-fold, from 23.5 cells/mm² to 85.1 cells/mm², while the number of Ia⁺, FVIII-RAg⁺ capillary endothelial cells increased 6.5-fold, from 3.6 cells/mm² to 23.5 cells/mm² (Fig. 1). On day 7 after injection, the class II expression had decreased back to the original level. In rat kidney the response was similar though less pronounced in intensity. In rat liver, the response of the DC to IFN-γ was even smaller (not shown); on the other hand, the response of liver endothelial cells to IFN-γ, in particular in the central vein area, was far more pronounced than in either heart or kidney, with an increase of Ia⁺, ink⁻, FVIII-RAg⁺ capillary endothelial cells from 5.4 cells/mm² to 126.7 cells/mm² (Fig. 1).

After injection of IFN-γ, the number of liver Ia⁻ phagocytic cells, most of which were present in the central vein area, decreased, and the number of Ia⁺ phagocytic cells increased, indicating an in situ activation of Kupffer cells (not shown).

Administration of a single bolus of 300 mg/kg of MP together with 10^5 U/kg of IFN-γ entirely abolished the effects of IFN-γ on endothelial cells and DC in all three organs (Fig. 1).
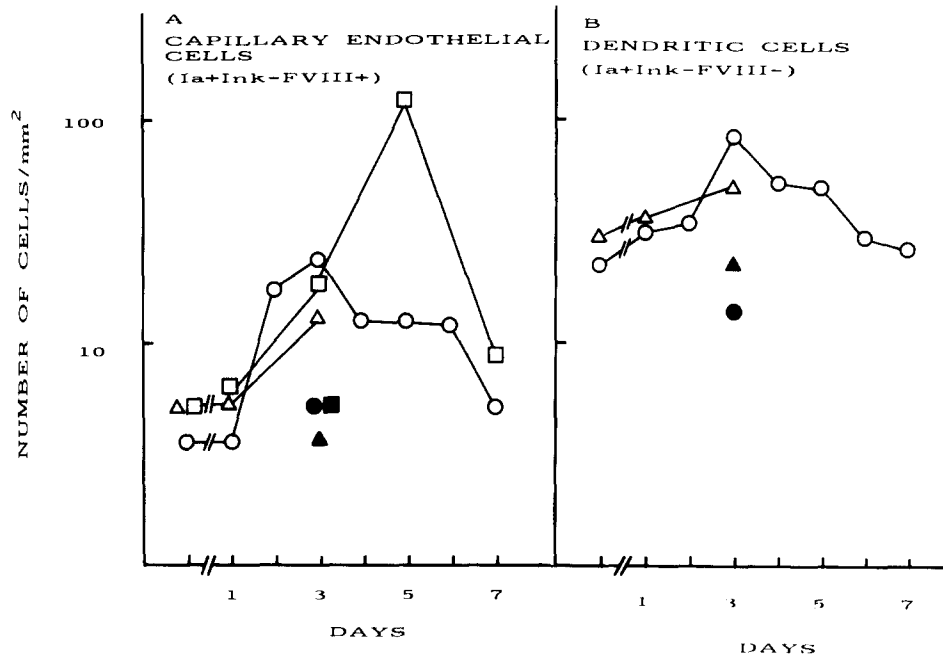
FIGURE 1.   Effect of 10^5 U/kg of rIFN-γ (open symbols) on class II expression in capillary endothelial cells (A) and on the density of tissue DC (B) in rat heart (circles), kidney (triangles), and liver (squares). The impact of concomitantly administered bolus of 300 mg/kg MP (filled symbols) is also shown. Note logarithmic scale.

The antagonistic effects of steroids on class II expression by endothelial cells and on the density of tissue DC were investigated further by using different doses of MP and different schedules of administration (Tables I and II).

A single bolus administration of MP was rather inefficient: 300 mg/kg of MP was needed to reduce the baseline expression of class I antigens on the endothelial cells by 30% and the baseline density of DC by 60–70%, whereas 30 mg/kg had no effect either on baseline or on IFN-γ-induced class II expression in rat heart and kidney (Fig. 1 and Table I). The liver DC and endothelial cells were even more resistant in both respects (Fig. 1, Table II).

On the other hand, repeated small doses were highly efficient. The baseline expression of class II on the capillary endothelial cells and the density of tissue DC were reduced in rat heart and kidney down to a dose range of 1–3 mg/kg/d, the kidney cells being somewhat more resistant than the heart cells. In the liver, the baseline class II expression of capillary endothelial cells was practically abolished at a dose of 1 mg/kg/d; the liver DC were more resistant to steroids than were the DC in the kidney and heart. A complete inhibition of IFN-γ induced class II expression on the endothelial cells, and an abolishment of IFN-γ-induced increase in DC density was obtained by 1 mg/kg/d of MP in all three sites of assay.

Discussion

The DC in this study were defined as strongly class II–positive nonphagocytic cells, which did not display the FVIII-RAg characteristic to the vascular endo-

TABLE I

Impact of IFN-γ and/or MP on Expression of Class II Antigen in DA Rat Heart and Kidney on Day 3 after Drug Administration

| | | Number of Ia-expressing cells/mm^2* | | | |
| | | DC Ia$^+$, ink$^-$, FVIII$^-$ | | Capillary EC Ia$^+$, ink$^-$, FVIII$^+$ | |
Drug	Dose	Heart	Kidney	Heart	Kidney
None	—	40.7	57.9	8.1	9.0
rIFN-γ‡	10^5 U/kg	55.2	74.2	21.7	21.7
rIFN-γ MP§	10^5 U/kg 30 mg/kg × 1	46.2	65.2	7.2	12.6
rIFN-γ MP	10^5 U/kg 30 mg/kg × 3	8.1	21.7	0.9	1.8
rIFN-γ MP	10^5 U/kg 10 mg/kg × 3	19.9	36.2	3.6	5.4
rIFN-γ MP	10^5 U/kg 3 mg/kg × 3	34.4	48.9	5.4	7.2
rIFN-γ MP	10^5 U/kg 1 mg/kg × 3	37.1	54.3	5.4	7.2
MP	300 mg/kg × 1	14.5	23.5	5.4	5.4
MP	30 mg/kg × 1	41.6	57.9	6.3	10.9
MP	30 mg/kg × 3	10.0	19.9	0.9	3.6
MP	10 mg/kg × 3	19.0	30.8	2.7	3.6
MP	3 mg/kg × 3	24.4	39.8	1.8	7.2
MP	1 mg/kg × 3	30.8	47.1	5.4	9.0

* The rats received 0.5 ml of 20% India ink in PBS 2 h before killing. The tissues were processed for frozen section histology and stained with mAb detecting the class II backbone, MAS029, using the double indirect immunofluorescence method with rabbit anti-FVIII-RAg (18). EC, endothelial cells.

‡ The most effective dose of rIFN-γ 10^5 U/kg, was pretested using a logarithmic scale from 10^3 U/kg upwards. The lowest dose giving a maximal response was selected.

§ MP was given at the indicated dose either together with rIFN-γ on day 0 (× 1) or on days 0, 1, and 2 (× 3) after rIFN-γ injection. All drugs were given i.p.

thelial cells. Tubular cells expressing class II antigens were easily distinguishable on morphological grounds. The remaining parenchymal structures in these organs did not express any detectable amounts of class II (9–11), and the presence of lymphoid T or B cells in tissue was excluded by proper antibodies. The

TABLE II

Effect of IFN-γ and/or MP on Expression of Class II Antigens in DA Rat Liver on Day 3 after Drug Administration

Drug*	Dose‡	Ia-expressing cell in central vein area§						Ia-expressing cells in portal area§	
		DC (strongly Ia+, ink−, FVIII−)	Kupffer cells + Mφ			Endothelial cells		DC (strongly Ia+, ink−, FVIII−)	Portal vein endothelium (Ia+, ink−, FVIII+)
			Strongly Ia+, ink+, FVIII−	Weakly Ia+, ink+, FVIII−	Ia−, ink+, FVIII−	Central vein (Ia+, ink−, FVIII+) %	Capillary (Ia+, ink−, FVIII+) %		%
		cells/mm²				%		*cells/mm²*	%
Normal rat	—	2	12	20	521	<5	15	470	<5
rIFN-γ (10⁵ U/kg)	—	2	27	80	430	25	100	770	25
rIFN-γ (10⁵ U/kg) + MP	30 mg/kg × 1	2	20	48	500	0	25	623	0
	30 mg/kg × 3	0	2	7	580	0	0	120	0
	10 mg/kg × 3	0	5	11	577	0	0	258	0
	3 mg/kg × 3	0	10	12	560	0	15	438	0
	1 mg/kg × 3	1	10	17	540	<5	15	523	<5
MP	300 mg/kg × 1	2	5	22	600	0	0	500	0
	30 mg/kg × 1	1	14	19	533	0	0	469	0
	30 mg/kg × 3	2	3	6	572	0	0	103	0
	10 mg/kg × 3	0	4	8	580	0	0	260	0
	3 mg/kg × 3	1	7	9	579	0	<5	380	0
	1 mg/kg × 3	1	10	14	582	<5	<5	433	<5

* The IFN-γ (10⁵ U/kg) dose was pretested with induction of Ia expression in rat heart (see Table I). The presence of T and B cells in the liver was excluded by lack of reactivity with antibodies MAS010 and MAS056 (Sera-Lab) respectively. The presence of granulocytes was excluded morphologically. Mφ, macrophage.

‡ Administration of MP: 30 mg/kg × 1 indicates that the drug was given simultaneously with rIFN-γ; 1–30 mg/kg × 3 means that the drug has been given at indicated dose on the day of rIFN-γ injection, and on days 1 and 2 as well.

§ The rats received 0.5 mg of 20% India Ink 2 h before they were killed. The number of Ia-expressing DC and Kupffer cells are given in cells/mm². The number of FVIII-RAg+ capillary endothelial cells are expressed in percent, as exact quantitation of Ia-expressing vascular endothelial cells is not possible to perform in the liver with planometric methods. The level of expression in vessel endothelium has been estimated from the double-stained preparations as <5%, 15, 25, or 100%.

localization and appearance of the DC in tissue and their properties (i.e., lack of phagocytosis) are the same as those reported previously by Hart and Fabre (7, 9), who also demonstrated their cell surface and histochemical phenotype and their bone marrow origin. We consider it likely, therefore, that most of the cells defined as dendritic cells in this study are indeed dendritic cells.

The tissue macrophages, on the other hand, were defined by their phagocytic ability and by their ingestion of intravenously administered India ink. As the possibility remains that India ink did not reach all phagocytic cells, the number of tissue macrophages (and Kupffer cells) may be slightly underestimated and that of the DC slightly overestimated. However, those cells that did ingest India ink may definitely be considered tissue phagocytes.

It has been demonstrated previously by others (14–19) that IFN administration in vitro upregulates class II expression on, e.g., human umbilical cord endothelial cells and in many cell lines of nonendothelial origin (14–16). The present in vivo results confirm the earlier in vitro findings by demonstrating that recombinant rat IFN-γ induces the expression of class II antigens on the (capillary) endothelial cells of rat heart, kidney, and liver. In addition, our results show that the number of Ia^+, $FVIII-RAg^-$ DC in rat tissue increases in response to IFN-γ. Both responses are abolished by administration of glucocorticosteroids.

Two modes of steroid administration were used: a single bolus administration on day 0, mimicking the pretreatment of the graft donor to reduce allograft immunogenicity, and repeated administration of small doses, mimicking the treatment of allograft rejection. Using single-bolus administration, ~300 mg/kg was required to reduce the density of DC and class II expression in the endothelial cells in rat heart and kidney. This dose is in the same dose range as the minimal dose of MP previously reported (20) to be effective in donor pretreatment in the rat. The liver DC were more resistant. On the other hand, repeated small doses were highly efficient: a distinct effect was obtained both on baseline and on IFN-γ-induced class II expression on the endothelial cells and on the density of tissue DC at a dose of 1–3 mg/kg/d. This dose is less than one-tenth the dose of MP used in most centers for the treatment of acute allograft rejection in man.

The impact of IFN administration on class II antigen expression by rat vascular endothelial cells was directed in particular to the capillary endothelium, and is most likely a direct one. In contrast, the antagonistic effect of MP may be indirect, as we have not been able to downregulate Ia antigen expression of endothelial cells by MP in vitro (our unpublished results).

The mechanism of regulation of tissue DC number is less clear. First, it is possible that some dormant DC, which do not display class II at a given time, exist in the tissue. This possibility is unlikely, as it goes against the definition of a DC lineage (21). The second possibility is that the new cells are derived from a pool of recirculating DC. This may be the case if IFN-γ has a direct effect on the permeability of the vascular wall and thus opens a gate through the vascular endothelial barrier. This interpretation is also compatible with the inhibitory effect of steroids. Unfortunately, the experimental conditions we used cannot distinguish between these two alterations. In our view, it is unlikely that these cells are newly synthesized DC from IFN-stimulated bone marrow, as it has been

shown previously (22, 23) that it takes 7–10 d for such cells to reach the tissue after bone marrow transplantation.

Two additional effects of IFN-γ administration, both of which were inhibited by steroids, were observed in this study. In the central vein area of liver, administration of IFN-γ was accompanied by a decrease in the number of Ia⁻, ink⁺ cells, and a concomitant increase in the number of Ia⁺, ink⁺ cells, whereas in the portal area there was a decrease in number of Ia⁺, ink⁺ cells (no Ia⁻, ink⁺ cells are normally present in this location). The effect in the central vein area is likely to be explained by IFN-induced activation of mononuclear phagocytes, which is inhibited by steroids. We have, at present, no explanation for the latter phenomenon.

Summary

A single injection of 10^5 U/kg of recombinant rat IFN-γ increases the amount of tissue dendritic cells up to sixfold, and concomitantly induces the (capillary) endothelial cells to express class II MHC antigens. Both responses peak on the third day after IFN-γ injection, and the antigen expression returns to basic levels on day 7. Simultaneous administration of 1 mg/kg/d of methylprednisolone entirely abolishes both responses. These observations demonstrate, for the first time, that IFN-γ and steroids have antagonistic effects on class II MHC antigen presentation in tissue, and suggest that one immunosuppressive mechanism of glucocorticosteroids in organ transplantation is downregulation of graft antigenicity.

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