INDUCTION OF Ia ANTIGEN IN RAT EPIDERMAL CELLS AND GUT EPITHELIUM BY IMMUNOLOGICAL STIMULI

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Although the tissue distribution of Ia antigens is by no means ubiquitous, a wide variety of cell types has been shown to express them. Some of these cells, such as B cells, macrophages, activated T cells, and dendritic cells of thymus, spleen, and lymph nodes, have a clear association with the immune system, but there are no data to suggest that Ia-bearing cells of the gut epithelium, kidney, bronchi (1–4), and lactating mammary gland (5) play an immunological role. There is evidence (6) that Ia antigens are involved in antigen recognition by T cells and may, in fact, be products of Ir genes, so that the presence of Ia antigens on cells with no established immunological function raises the question of the role of Ia antigens in these sites. In principle, two answers seem possible. Either the Ia antigens in these apparently nonimmunological sites play essentially the same role as they do in the immune system, i.e., they serve as restriction elements in the recognition of antigen by T cells, or, alternatively, Ia antigens have functions in addition to those related to the immune system.

Recently, it has been shown that when graft-vs.-host disease (GvHD)[1] is induced in irradiated F_1 hybrid rats by the injection of parental lymphocytes, large amounts of intracellular Ia antigens appear in epidermal cells and in the cells of the gut epithelium (7, 8). The work presented here extends these preliminary findings and demonstrates that naturally occurring immunological stimuli can also induce the expression of Ia antigens at these sites. These observations support the idea that this inducible Ia has an immunological role. The implications of this suggestion are discussed.

Materials and Methods

Animals. Inbred strains PVG.RT1^c, DA.RT1^a, and their F_1 hybrids from our own specific pathogen-free unit were used for most experiments. For a few experiments, (PVG.RT1^c × PVG.RT1^u)F_1 hybrids served as recipients of PVG thoracic duct lymphocytes (TDL).

Cells. Preparation of spleen cells, bone marrow cells, and TDL followed established procedures (9).

Antibodies. The derivation of monoclonal antibodies W3/25 and MRC OX-8, which recognize T helper (T_h) and T cytotoxic/suppressor ($T_{c/s}$) subsets of rat T lymphocytes, respectively, is given in references 10–12. Monoclonal mouse anti-rat Ia antibodies were used to detect Ia antigen in cryostat sections; MRC OX-6 or MRC OX-4, which recognize the same nonpolymorphic determinant of the rat homologue of mouse I-A (13), MRC OX-17, which recognizes the rat homologue of mouse I-E (14), and F-17-23-2, which recognizes Ia of DA but not PVG inbred strains (3). The monoclonal antibodies were used as tissue culture supernatants

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[1] *Abbreviations used in this paper:* DAB, Dulbecco's medium A + B; GvHD, graft-vs.-host disease; IL-1, interleukin 1; MHC, major histocompatibility complex; NCS, newborn calf serum; TDL, thoracic duct lymphocytes; T_h, T helper cell; $T_{c/s}$, T cytotoxic/suppressor cell.

from the hybridomas, except for F-17-23-2 antibody, which was IgG prepared from ascitic fluid (3) and used at 20 μg/ml.

Induction of Graft-vs.-Host Disease. TDL from PVG rats were washed twice in Dulbecco's A + B medium (DAB) containing 2% newborn calf serum (NCS) and injected into F_1 hybrid recipients that had received 450 rad irradiation from a ^{137}Cs source a few hours previously. The cell doses used are given in Results.

To induce GvHD in rats that were bone marrow chimeras, (PVG $\times$ DA)F_1 hybrid rats were given 950 rad ^{137}Cs irradiation, followed by intravenous injection of 10^7 PVG bone marrow cells. 13 d later, they were injected intravenously with 10^8 PVG spleen cells.

Cell Fractionation. Parental strain TDL were depleted of T_h or $T_{c/s}$ subsets of T cells by a modification (15) of the rosetting technique of Parish and Hayward (16) using the monoclonal antibodies W3/25 and MRC OX-8. Details of the methods and the determination of the purity of the depleted populations, by flow cytofluorimetry, are given in ref. 15.

Detection of Ia Antigen in Cryostat Sections. Cryostat sections of skin or small intestine were stained for the presence of Ia.antigen using MRC OX-6, MRC OX-4, MRC OX-17, and F-17-23-2 monoclonal antibodies by means of an immunoperoxidase technique (17). The procedure used to eliminate nonspecific binding of the antibodies to skin sections is described in ref. 8. Monoclonal antibodies W6/32 and W3/13 (10, 17), which do not react with rat Ia antigen, were used as specificity controls. In some cases, sections were lightly counterstained with Harris' hematoxylin.

Preparation and Staining of Peritoneal Macrophages. Peritoneal macrophages from both normal and F_1 hybrid rats and those undergoing GvHD were obtained by flushing out the peritoneal cavity with DAB/2% NCS. Smears made of the recovered cells were stained for the presence of Ia antigen and W3/25 antigen by the same immunoperoxidase technique as that used on cryostat sections of tissues. To aid in the unambiguous identification of macrophages on these smears, donor rats were injected intraperitoneally with 2 ml of a 0.1% suspension of latex particles 20 min before peritoneal wash-out, and only cells containing phagocytosed latex were scored for the presence of Ia antigen.

Radioimmunoassay for the Detection of Ia Antigen in Rat Serum and Lymph. Ia antigen was quantitated in serum and lymph from normal rats and from rats undergoing GvHD by inhibition of the binding of MRC OX-6 antibody to TDL in an indirect radioactive binding assay, as described previously (13). In brief, a 50-μl sample was incubated with 50 μl MRC OX-6 tissue culture supernatant diluted 1/200 for 1 h at 4°C. This large dilution of tissue culture supernatant was chosen to maximize sensitivity. 2.7 $\times$ 10^6 TDL were added and incubated for 1 h. The target cells were washed and incubated with ^{125}I-labeled F(ab')$_2$ rabbit anti-mouse IgG (50 μl, 0.15 μg/ml, 15 μCi/μg) for 1 h at 4°C before washing and counting. The assay was calibrated using Ia antigen purified from rat spleen by monoclonal antibody affinity chromatography (13).

Contact Sensitization and Challenge with Dinitrofluorobenzene. Normal (PVG $\times$ DA)F_1 hybrid rats were painted on the abdomen with 100 μl of a 0.5% solution of dinitrofluorobenzene dissolved in a 4:1 mixture of acetone in olive oil. Starting 7 d later, they were challenged on the ears for 4 successive days with a 0.2% solution of dinitrofluorobenzene in the same medium. Ear skin was taken for cryostat sections 24 h after the last challenge and stained for the presence of Ia antigens using MRC OX-4 and MRC OX-6 monoclonal antibodies.

Infection of Rats with the Parasite Trichinella spiralis. The strain of *T. spiralis* used was kindly donated by Dr. M. Phillippe, Division of Parasitology, National Institute for Medical Research, Mill Hill, London, England. The preparation of *T. spiralis* larvae and injection of rats were as described by Love et al. (18). Male PVG rats aged 3 mo were infected intragastrically with 500 or 1,000 *T. spiralis* larvae, and samples of small intestine $\sim$5 cm from the pylorus were taken for localization of Ia antigens on cryostat sections. In two independent time-course experiments, pairs of rats were sampled for immunohistochemistry at 2, 4, 8, 12, 16, 20, 24 d after injection.

Results

Both T_h and $T_{c/s}$ Subsets of Parental TDL Induce Ia Antigen Expression in Gut and Skin of F_1 Recipients. Previous experiments (8), showing that parental TDL injected into

sublethally irradiated F_1 recipients induced the expression of Ia in epidermal cells and gut epithelium, used unfractionated inocula of parental cells. To determine whether parental T cells were essential for this induction and to see whether the T_h or the $T_{c/s}$ subset was involved, parental TDL were depleted of one or the other or both T cell subsets, and the residual cells were injected into irradiated F_1 hybrids. As Table I shows, simultaneous depletion of both T cell subsets rendered the donor

TABLE I

Both T_h (W3/25$^+$) and $T_{c/s}$ (MRC OX-8$^+$) Subsets of Parental T Cells Induce the Expression of Ia Antigen in Epidermis and Gut Epithelium of F_1 Recipients

Experi-ment	PVG TDL transferred	Recipient	Ia induced	
			In epider-mis	In gut epi-thelium
1	6×10^7 unfractionated	(PVG $\times$ DA)F_1	++	ND
	3.7×10^7 depleted of $T_{c/s}$		++	ND
2	6×10^7 unfractionated	(PVG $\times$ DA)F_1	ND	++
	6×10^7 depleted of $T_{c/s}$		ND	++
	6×10^7 depleted of $T_{c/s}$ and T_h		ND	—
	2.4×10^6 unfractionated		ND	—
3	6×10^7 unfractionated	(PVG $\times$ PVG.RT1^u)F_1	ND	++
	6×10^6 unfractionated		ND	—
	6×10^5 unfractionated		ND	—
	6×10^7 depleted of T_h		ND	++
	6×10^6 depleted of T_h		ND	—
	6×10^5 depleted of T_h		ND	—
4	5×10^7 depleted of T_h	(PVG $\times$ DA)F_1	ND	++
	5×10^7 depleted of T_h and $T_{c/s}$		ND	—
5	4.3×10^7 unfractionated	(PVG $\times$ DA)F_1	++	ND
	7×10^7 depleted of T_h		++	ND

Experiment 1: PVG TDL were depleted of MRC OX-8$^+$ cells by rosetting. By FACS analysis, there were 6.7% MRC OX-8$^+$ cells present before depletion and 0.09% after depletion. Ear skin was taken for immunochemical staining for Ia antigen on days 12 and 17 after cell transfers. On day 12, the epidermis of the recipient of unfractionated TDL was strongly positive for Ia, but that of the recipients of the TDL depleted of $T_{c/s}$ was less strongly stained. However, by day 17 the epidermis of recipients of depleted TDL were strongly Ia positive.
Experiment 2: By FACS analysis, there were 7.5% MRC OX-8$^+$ cells and 42.2% W3/25$^+$ cells in the unfractionated TDL. After depletion of $T_{c/s}$ the residual MRC OX-8$^+$ cells were undetectable (<0.05%), while after the combined depletion of T_h and $T_{c/s}$, 0.21% cells labeled with a mixture of W3/25 and MRC OX-8 monoclonal antibodies. Tissues were taken for staining on day 13. The failure of 2.4×10^6 unfractionated TDL to induce the expression of Ia in the gut epithelium shows that the positive result obtained with the inoculum depleted of $T_{c/s}$ was not due to inadequacy of the depletion procedure.
Experiment 3: There were 43.1% W3/25$^+$ cells and 7.6% MRC OX-8$^+$ cells in the unfractionated TDL. After depletion, no T_h cells were detectable, while the $T_{c/s}$ population had increased to 13.0%. The dose-response result obtained with unfractionated TDL indicates that even a 4.3% contamination of the depleted population with W3/25$^+$ cells would not account for the observed induction of Ia in the recipients of depleted TDL. Cryostat sections were prepared on day 15 after cell transfer.
Experiment 4: The undepleted TDL contained 38.2% W3/25$^+$ cells and 6.4% MRC OX-8$^+$ cells. By FACS analysis, both depletions reduced the percentage of labeled cells to <2%, and the depletion of W3/25$^+$ cells increased the percentage of MRC OX-8$^+$ cells to ~9.5%. Tissues were taken for histology on day 12.
Experiment 5: Depletion of TDL of W3/25$^+$ cells reduced the percentage of T_h cells from 43.3% to 0.1%. Ear skin was taken for cryostat sections on day 13 after cell transfer.

inoculum incapable of inducing Ia expression in the F_1 recipients, and, as expected, such recipients did not develop signs of GvHD (15). However, recipients of parental TDL depleted of either the T_h subset alone or the $T_{c/s}$ subset alone did express intracellular Ia in epidermal cells and gut epithelium. Experiments carried out to exclude the possibility that inadequacy of the depletion procedure used to remove one or other of the T cell subsets from the donor inoculum was responsible for these results only confirmed that both functional subsets were capable of inducing Ia in the F_1 hosts (Table I).

Previous experiments (15) have established that both T cell subsets are able to mediate GvHD, but whether the induction of epidermal and gut Ia plays any role in pathogenesis is unknown.

Expression of Ia Antigen on Peritoneal Macrophages during GvHD. The induction of GvHD in rodents leads to the activation of macrophages, as shown by the relative resistance of such animals to infection with *Listeria* (19). This organism is effectively killed by activated macrophages but not by nonactivated ones. When the macrophages from irradiated F_1 hybrid recipients of 5×10^7 parental TDL were examined for the expression of Ia antigen, >98% were found to be positive by day 10 after injection. This figure contrasts with the 10% that are Ia^+ in normal rats (17) or irradiated F_1 rats injected with medium alone. As with the induction of epidermal and gut epithelial Ia antigen, both T_h and $T_{c/s}$ subsets of parental cells induced the appearance of Ia^+ macrophages. The numbers of macrophages recovered from the peritoneal cavity of rats suffering from GvHD ($5 \times 10^6-10^7$/rat) were not significantly different from those obtained from normal rats.

Ia Antigen Induced by GvHD Is of Host Origin and Is Not Acquired from Bone Marrow-derived Cells. When cryostat sections of ear skin and small intestine obtained from rats suffering from GvHD were labeled with mouse monoclonal antibody F-17-23-2, reactive with Ia antigen of the unshared haplotype between donor and F_1 host, the epidermal cells of the skin and the epithelial cells of the gut were clearly positive. Although this experiment does not, in principle, rule out a contribution from the donor for the intracellular Ia found in the host cells, it does show that at least some of the Ia was of host origin.

To determine whether the gut epithelial Ia and epidermal Ia were acquired from bone marrow-derived cells, studies were carried out using chimeric animals. Within 12 d of the injection of PVG spleen cells into chimeric rats, prepared by injecting lethally irradiated (PVG × DA)F_1 rats with PVG bone marrow, recipient rats developed the erythema and hunched posture characteristic of GvHD. Cryostat sections of the small intestine and ear skin of one of these rats, made 14 d after spleen cell injection, were stained with monoclonal antibody F-17-23-2 or with a monoclonal antibody, MRC OX-6, which recognizes a monomorphic determinant of rat Ia. As Fig. 1 shows, both the lamina propria macrophages and the gut epithelium were labeled by the MRC OX-6 antibody, but only the gut epithelium was labeled with F-17-23-2. It appears that the lamina propria cells were entirely of bone marrow donor origin and that the Ia in the gut epithelium did not derive from these cells. The experiment also establishes the important finding that the lamina propria macrophages did not take up detectable amounts of Ia from the gut epithelial cells. The results obtained from the skin sections closely paralleled those of the gut in that the epidermal cells stained with both F-17-23-2 and MRC OX-6 antibodies, but Ia^+ cells
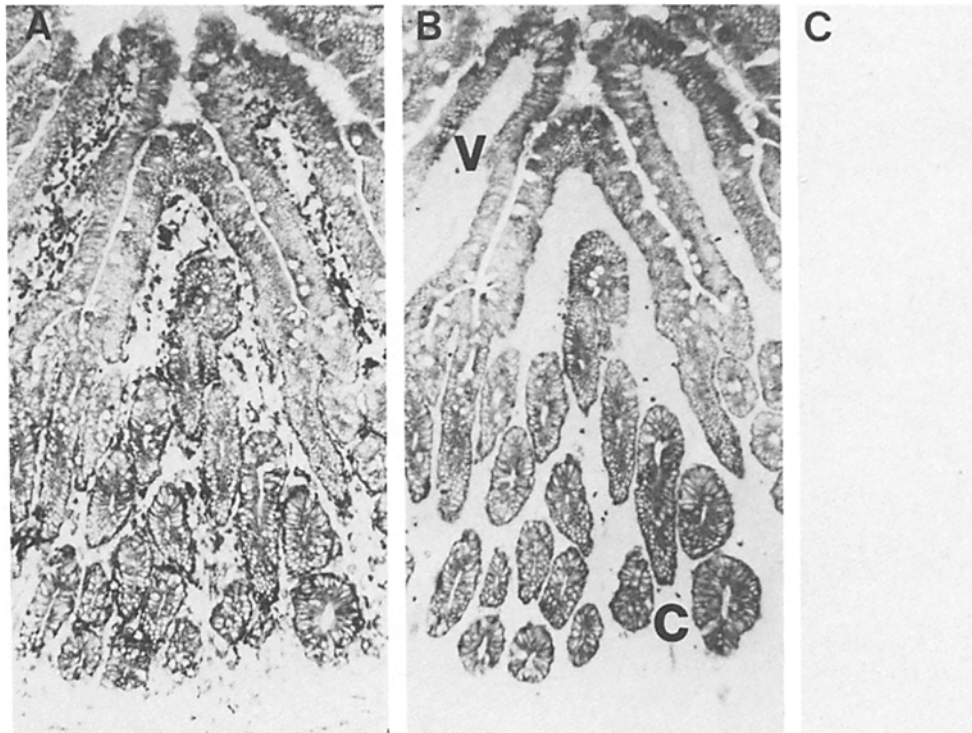
Fig. 1. Ia antigen induced by GvHD is not acquired from bone marrow-derived cells. (PVG × DA)F₁ rats were lethally irradiated and given 10^7 PVG bone marrow cells intravenously. 13 d later, they were injected intravenously with 10^8 PVG spleen cells, and 2 wk after this injection cryostat sections of ear skin and small intestine were stained for the presence of Ia antigen. (A) Small intestine of chimeric rat with GvHD stained with MRC OX-6 monoclonal antibody (monomorphic determinant of Ia). The villus epithelium and the lamina propria macrophages are stained. (B) Section from the same block as (A) but stained with F-17-23-2 monoclonal antibody (reactive with host Ia only). The epithelium only is stained. (C) Control section from the same block as (A) stained with W6/32 antibody (not reactive with rat tissues). The rare positive cells are probably eosinophils that stain nonspecifically with the peroxidase reagent. v, villus; c, crypt.

in the dermis reacted only with the latter antibody (data not shown).

Failure to Detect Ia Antigen in Serum of Rats with GvHD. It has been argued that the Ia antigen found in the epidermis and gut epithelium of rats with GvHD was produced by the cells in which it was found (8). In the epidermis in particular there are, apart from the relatively sparse Ia⁺ Langerhans cells, no Ia⁺ cells that could serve as a local source of Ia. If the epidermal cells acquired Ia from an extracellular source, then the only possible candidate appears to be the serum. Accordingly, the serum of rats with GvHD was assayed for the presence of Ia antigen using a radioimmunoassay. As Fig. 2 shows, if such serum contains Ia antigen, it lies below the level of detection of the assay, i.e., < 50 ng/ml. In view of claims that Ia antigens are present in mouse serum (20), it is significant that this assay also failed to detect Ia in normal rat serum.

The most likely conclusion from these two experiments is that the epidermal and gut epithelial Ia induced by GvHD is of host origin and is synthesized by the cells containing it.

Induction of Ia in Gut Epithelium by Infection of the Gut Lumen with the Parasite T.
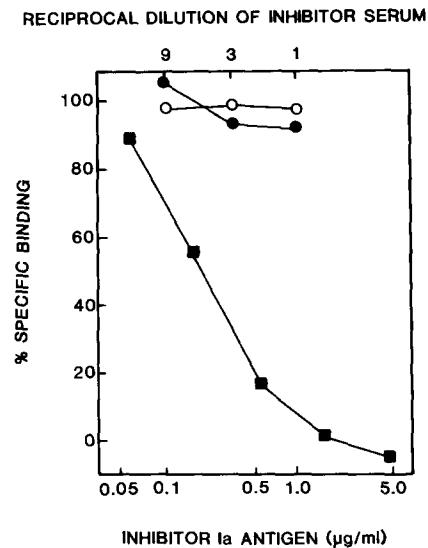
RECIPROCAL DILUTION OF INHIBITOR SERUM

FIG. 2. Quantitation of Ia antigen in rat serum from normal rats and rats undergoing GVH. The Ia antigen content of normal rat serum (O), serum from a rat 12 d after induction of GVH (●), and purified Ia antigen [■] were quantitated by inhibition of an indirect radioimmunoassay for Ia antigen. 100% binding represents 3,050 cpm obtained with MRC OX-6 antibody plus 2% NCS serum alone with 850 cpm for background (^{125}I antibody alone) subtracted.

spiralis.   GvHD is not a condition encountered by animals in their natural environment. It was of interest, therefore, to determine whether the expression of Ia antigen in gut epithelium could be induced by a more normal immune stimulus. PVG rats were infected with *T. spiralis* larvae and Ia antigen localized subsequently on cryostat sections of small intestine. Ia antigen is normally present in low amounts in the villus epithelium in the strain of rats used but is never seen in the crypt epithelium.[2] Between days 8 and 12 postinfection, Ia antigen was clearly present in the crypt epithelium (Fig. 3), which coincides with the main period of expulsion of worms (18). Ia antigen expression in the villus epithelium increased at 12–16 d postinfection, i.e., somewhat later than induction in the crypts.

The lower part of the villus epithelium and the majority of the crypt epithelium except that close to the muscularis did not stain for Ia antigen, indicating that the expression of Ia antigen can be lost as epithelial cells migrate from the crypts to the villus, where it can be reexpressed. Attempts to reinduce Ia expression in gut epithelium by secondary challenge of rats that had been primed 34 d earlier by intragastric administration of larvae were unsuccessful, possibly because the larvae are promptly expelled from the gut (18).

Contact Sensitization with Dinitrofluorobenzene Induces Expression of Ia in Epidermal Cells.   When contact sensitivity to dinitrofluorobenzene was induced by skin painting, followed 7 d later by challenge of the ear skin with the same reagent, cryostat sections of the challenged skin showed Ia$^+$ epidermal cells (Fig. 4). As with the Ia antigen in the gut induced by *T. spiralis* infection, the staining was patchy and generally weaker

[2] Mayrhofer, G., C. W. Pugh, and A. N. Barclay. The distribution, ontogeny, and origin in the rat of Ia-positive cells with dendritic morphology and of Ia antigen in epithelia with special reference to the intestine. Manuscript submitted for publication.
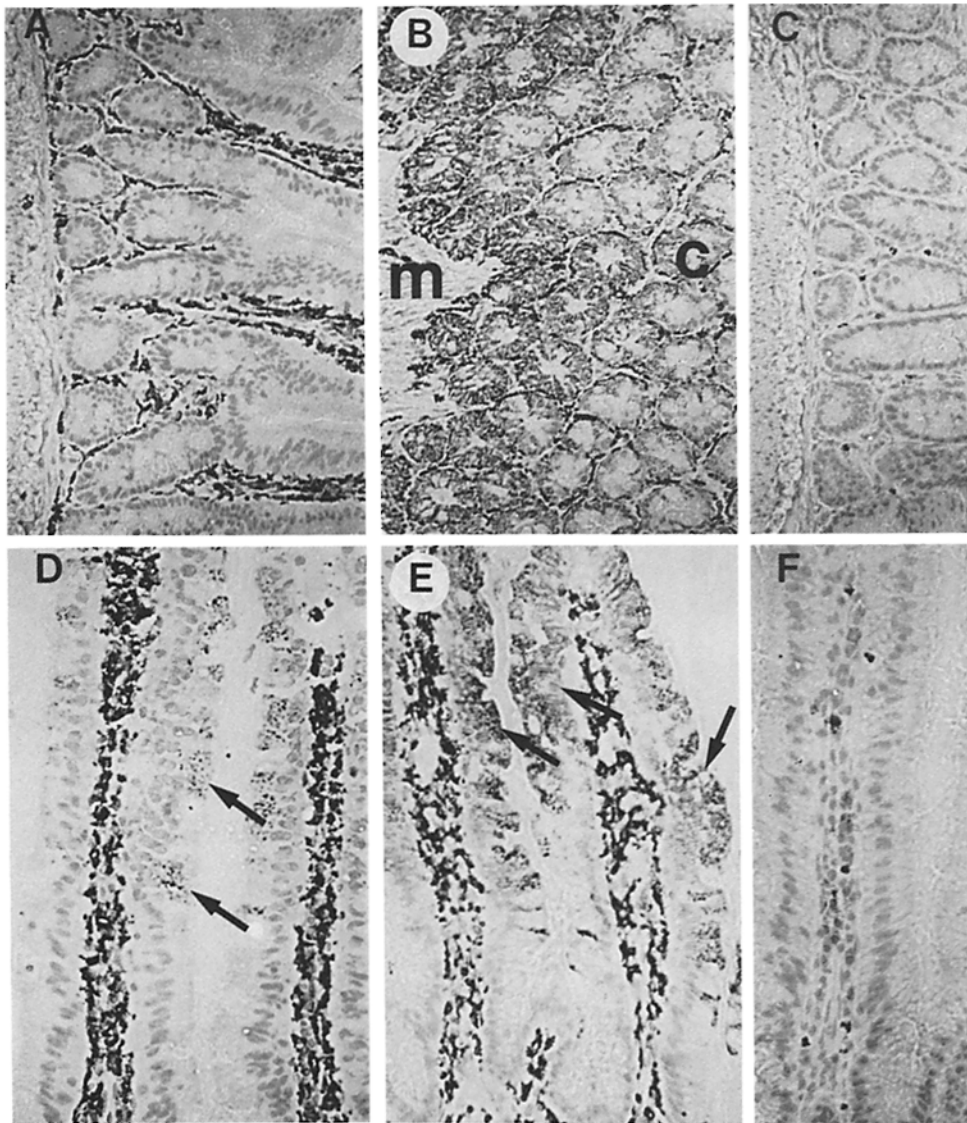
FIG. 3. Induction of Ia antigen in epithelia of small intestine during infection with *Trichinella spiralis*. Ia antigen was localized on cryostat sections by immunoperoxidase staining. (A) Normal rat; MRC OX-4 antibody stains cells in the lamina propria and between the crypts but not the crypt epithelium. (B) Rat 8 d after oral infection with 500 *T. spiralis* larvae. MRC OX-4 antibody stains the crypts (c) especially those adjacent to the muscle (m). (C) Normal rat; W6/32 antibody shows low levels of nonspecific staining. Scattered stained cells are probably eosinophils. (D) Normal rat; MRC OX-4 antibody gives heavy staining of cells in the lamina propria and weaker, granular staining of epithelium in the distal ⅔ of the villi (arrow). (E) Rat 16 d after oral infection with 500 *T. spiralis* larvae. MRC OX-4 antibody gives heavier staining of the epithelial cells of villi than in normal rats (arrows) (see D). (F) Normal rat; W6/32 antibody shows low levels of nonspecific staining. In D, E, and F the bases of the villi are at the bottom of the figure. All sections are counterstained with hematoxylin and viewed under phase contrast.
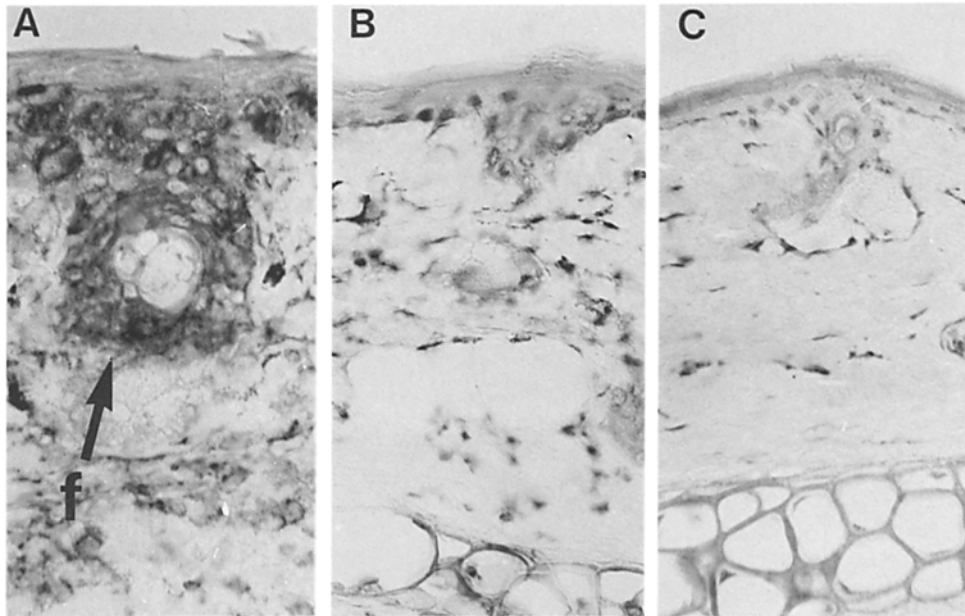
Fig. 4.   Induction of epidermal Ia antigen by contact sensitization. Contact sensitivity to dinitro-fluorobenzene was induced in (PVG × DA)F₁ hybrid rats. Cryostat sections of ear skin were taken for immunohistochemical staining for Ia 24 h after final challenge. (A) Ear skin from sensitized rat stained for Ia using MRC OX-4 monoclonal antibody. (B) Ear skin of sensitized rat stained with W3/13 monoclonal antibody that reacts with rat T cells, polymorphs, and brain. (C) Same as (A) except that ear skin was obtained from an unsensitized (PVG × DA)F₁ donor. All sections counterstained with hematoxylin and include part of a hair follicle (f).

than that seen in GvHD, where the staining was heavy and continuous over the epidermis (8).

Intestinal Lymph of Rats with GvHD Contains No Detectable Ia Antigen.   The observation that Ia antigen could be induced in epidermis and gut epithelium by more natural immunological stimuli than GvHD suggested that the induction had some immunological role. One possibility considered was that the Ia antigen induced might complex with antigen in these sites and travel via the lymph to draining lymph nodes. Intestinal lymph was obtained by thoracic duct cannulation of F₁ hybrid rats that had been mesenteric lymphadenectomized (21) 8 wk before the induction of GvHD and this lymph assayed for the presence of Ia antigen using the same protocol as that used for the detection of Ia in serum. No Ia was detectable.

Discussion

There can be little doubt that the antigen induced in epidermis and gut epithelium by GvHD is rat Ia. Four different monoclonal antibodies, two against a monomorphic determinant on the rat equivalent of mouse I-A, one against a monomorphic determinant of rat I-E/C and one against a polymorphic determinant of rat Ia, all labeled the putative Ia antigens. Although only some of these antibodies were used to define epidermal Ia induced by contact sensitization with dinitrofluorobenzene and gut epithelial Ia induced by *T. spiralis* infection, it seems most unlikely that in these cases the antibodies used were detecting hitherto unrecognized cross-reacting antigens.

These conclusions are supported by the biochemical evidence for Ia glycoprotein in guinea-pig gut epithelium (1).

The finding that parental inocula depleted of T cells failed to induce Ia in irradiated F_1 recipients is best explained by the assumption that induction of Ia occurred only when T cells in the donor inoculum were present to interact with transplantation antigens in the host. An unexpected finding was that both T_h and $T_{c/s}$ subsets of parental T cells were able to induce Ia expression. In all other assays of T cell function, the T cell subset defined by W3/25 monoclonal antibody has played a regulatory role (T_h for B cells and for T_c cells), whereas the MRC OX-8$^+$ subset has been shown to contain the precursors of T_c and T_s cells (11, 12, 22). However, it has been found that both T cell subsets alone are able to mediate lethal GvHD (15). There is evidence that, in the mouse, Ly-2$^-$ and Ly-2$^+$ cells can both have regulatory and effector T cell functions but that the role played by either subset is determined by whether the antigen recognized is K, D, or Ia (23). The observation that both rat T cell subsets induce the expression of host Ia during GvHD indicates that, for this activity, both subsets can perform the same function, but it is unknown at present whether the two T cell subsets are reacting with alloantigens coded for by different regions of the rat major histocompatibility complex (MHC).

The experiments using the monoclonal antibody that recognized host but not donor Ia showed that, at least for the allelic form that was not shared between host and donor, the Ia in the epidermis and gut epithelium of rats suffering from GvHD was of host origin. Furthermore, the experiment in which the disease was induced in bone marrow chimeras demonstrated that this Ia was not acquired from bone marrow-derived cells. These results, together with the failure to demonstrate Ia antigen in blood or lymph from rats with GvHD, strongly suggest that the Ia was synthesized by the cells of the epidermis and the gut epithelium and was not acquired from other cells. Although the origin of epidermal Ia induced by contact sensitization and of the Ia induced by *T. spiralis* infection in the crypt epithelium of the small intestine was not investigated, there is no reason to suggest that, in these situations also, the Ia was produced by cells other than those that expressed it. This conclusion is strengthened by the observation that Ia antigen found in the villus epithelium of normal rats has also been shown not to be acquired from bone marrow-derived cells.[2]

The Ia antigen expression in the epidermis and in the epithelium of the small intestine induced by these specific immunological stimuli was patchy rather than complete, and, in this respect, differed from that induced by GvHD. This irregular distribution suggests that induction is a consequence of local rather than systemic effects, a conclusion that is supported by the observation that the epidermis of skin allografts in the rat occasionally becomes Ia$^+$ before the grafts are rejected (M. Dallman, personal communication). It seems most reasonable to conclude that Ia induction in both the epidermis and gut epithelium occurs as a consequence of the local recognition of antigen by T cells. In this respect, these tissues resemble macrophages that can also be induced to express Ia by the products of activated T cells (24), and it is significant that, in the rats with GvHD, virtually all the peritoneal macrophages became Ia$^+$. It has been reported that interferon increases the expression of class I but not class II (Ia) MHC antigens on a variety of cell types (25–27). It appears that the level of expression of MHC antigen is labile and is influenced by products of the immune system.

Attempts to detect the transfer of the gut epithelial Ia induced by GvHD to the Ia^+ cells present in the lamina propria or to detect Ia antigen in the lymph draining the intestine were unsuccessful. These results suggest but do not prove that whatever the role of this Ia antigen might be, it is played out at the site of its induction rather than at some remote site, such as the lamina propria or the draining lymph nodes, and this probably also applies to the epidermal Ia. Because T cells are to be found in tissues and keratinocytes are reportedly able to synthesize the lymphokine interleukin 1 (IL-1) (28), it may be postulated that, for epidermis containing Ia^+ epidermal cells, the essential components for the activation of Ia-restricted T cells are present. If this is so, then the induction of Ia antigen in keratinocytes by contact-sensitizing agents and other immunological stimuli may enable T cell activation to take place with the keratinocytes playing the role of Ia^+, IL-1-producing, antigen-presenting cells. If a similar role is played by Ia^+ gut epithelial cells, it may be anticipated that these cells, like epidermal cells, will also produce IL-1. This possibility remains to be examined. In any event, the induction of Ia in epidermis and gut epithelium by immunological stimuli strongly supports an immunological role for its expression, and it is unnecessary to postulate that Ia antigens also have functions that are unrelated to the immune system.

Summary

The expression of Ia antigen in rat keratinocytes and gut epithelium was found to be inducible by a variety of immunological stimuli. Graft-vs.-host disease (GvHD) was accompanied by the appearance of Ia antigen in both sites, whereas local immunological stimuli, such as a contact-sensitizing agent applied to the skin and *Trichinella spiralis* infection of the gut, caused the expression of Ia antigen confined to the sites of contact of these stimuli with the tissues involved. Both T helper and T cytotoxic/suppressor subsets of parental lymphocytes, used to produce GvHD in F_1 hybrid recipients, induce Ia expression in the skin and gut of these hosts, but simultaneous removal of both subsets from the donor inocula prevented induction. The Ia antigen expression associated with GvHD was shown to be of host origin but was not acquired from bone marrow-derived cells. Attempts to detect Ia antigen in serum or lymph of rats with GvHD gave negative results, and it was shown that Ia^+ cells in the lamina propria of the small intestine did not take up detectable amounts of Ia antigen from the Ia^+ intestinal epithelium.

It appears that the local recognition of antigen by T lymphocytes can result in the induction of Ia antigen in keratinocytes and in the epithelial cells of the intestine. This antigen is synthesized by the cells in which it is found, and the observation that immunological stimuli are responsible for its appearance suggests that its role is an immunological one. Failure to find evidence that the gut epithelium Ia antigen was transferred to lymph or taken up by other Ia^+ cells in the intestinal villi supports the view that this Ia (and, by analogy, that found in keratinocytes) serves a local function, and the possibility that it is involved in antigen presentation to T cells is discussed.

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