MASSIVE INDUCTION OF DONOR-TYPE CLASS I AND CLASS II MAJOR HISTOCOMPATIBILITY COMPLEX ANTIGENS IN REJECTING CARDIAC ALLOGRAFTS IN THE RAT

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The major histocompatibility complex (MHC)[1] is normally involved in the regulation of T lymphocyte reactivity (1). In the physiologically abnormal situation of tissue transplantation, MHC alloantigens appear to be the major targets of the rejection response, at least in first-set responses. Both class I and class II alloantigens can induce rejection responses (2), and each class of antigen tends to induce different types of responses. Thus, although cytotoxic T lymphocytes of the T helper phenotype, which are specific for class II antigens, are well documented (e.g., reference 3), cytotoxic T lymphocytes appear to be mainly of the suppressor/cytotoxic phenotype and to be specific for class I antigens (4). Class II antigens appear to preferentially stimulate proliferative responses in T lymphocytes of the helper phenotype (5), resulting, in vivo, in delayed-type hypersensitivity (DTH) responses.

The contributions of T lymphocytes of the helper and suppressor/cytotoxic phenotypes to the rejection response are not entirely clear. T helper cells are certainly critical for the induction of effective rejection responses (6, 7). The contribution of each phenotype to the effector mechanisms of rejection is, however, controversial. On balance, it would seem likely that both cytotoxic T cells (directed mainly at class I antigens) and T helper cells (stimulated mainly by class II antigens for DTH reactions) contribute to the rejection of MHC-incompatible allografts (6, 7). The parameters of the rejection response provoked by an allograft depend on the expression of class I or class II MHC antigens in the graft tissue. The efficacy with which the response is provoked, the mechanism by which it arises, and the development and culmination of the reaction all depend on the expression and accessibility of class I or class II antigens within the graft.

The multitude of data indicating that the MHC expression is pleiotropic might therefore be of fundamental importance in transplantation. Many in vitro studies with α, β, and γ interferons have shown that these agents are potent inducers of class I MHC antigens on diverse cell types (e.g., 8, 9) with γ interferon also being

[1] *Abbreviations used in this paper:* BSA, bovine serum albumin; DTH, delayed-type hypersensitivity; MHC, major histocompatibility complex; PBS, phosphate-buffered saline; RAM, immunoadsorbent-purified rabbit F(ab')$_2$ anti-mouse F(ab')$_2$; RBC, red blood cell.

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a potent inducer of class II antigens (9, 10). To our knowledge, class I antigen induction has not been reported in vivo, but class II antigens have been induced by graft-vs.-host disease in skin and colonic epithelium (11, 12) and in kidney (13). Of particular interest is the induction of class II antigens on vascular endothelium (14) and epithelial cells (15) in rejecting skin allografts.

A characteristic of virtually all types of rejection responses is a prominent cellular infiltrate, with activated leukocytes of many types and the release of lymphokines within graft tissues. In such circumstances, donor MHC expression in the grafted tissues might be radically altered in the course of rejection, and this might play a critical role in the development and execution of the rejection response. For this reason, we have studied cardiac allografts for MHC expression at various stages during rejection, using immunohistological techniques and quantitative absorption analyses with allospecific mouse monoclonal xenoantibodies to rat class I and class II MHC antigens. We report a massive increase in class I expression by day 5 of the rejection process, mainly as a result of induction of class I antigens on myocardial cells (which normally express little or no class I antigen). Class II antigens, normally seen only on interstitial cells, appear in scattered areas of the myocardium by day 3, and by day 5 both endothelial and myocardial cells strongly express class II molecules.

Materials and Methods

Animals. Inbred DA ($RT1^a$) and PVG ($RT1^c$) male rats were obtained from OLAC 1976 Ltd., Bicester, Oxon., England, and were used between 3 and 6 mo of age.

Heart Transplantation. Heterotopic heart allografts were performed using microsurgical techniques. After the left kidney was removed, donor hearts were anastomosed to the recipient using end-to-end anastomoses between the renal and innominate arteries, and between the left branch of the pulmonary artery and the renal vein. Ischemia times were in the range of 40–50 min. Heartbeat was checked daily by palpation through the abdominal wall. Hearts were excised on days 1, 3, 5, and 7 after transplantation.

Tissue Homogenates. 50% tissue homogenates of normal DA hearts, and DA-to-DA isografted and DA-to-PVG allografted hearts (at day 5 after transplantation), were prepared. Hearts were removed and frozen immediately at −40°C until used. On thawing, the tissues were minced with scissors in Tris-buffered saline containing proteolytic inhibitors (2.5 mM iodoacetamide, 5 mM EDTA and 2 mM phenylmethylsulfonyl fluoride). The tissue was forced through a metal sieve and then mechanically homogenized with a Teflon pestle. The homogenate was washed twice in Tris-buffered saline by centrifuging at 13,000 *g* for 20 min, and the second pellet was resuspended in an equal volume of Tris-buffered saline and frozen at −40°C until used. All procedures were carried out at 4°C or on ice.

Cell Suspensions. Spleen cell suspensions were prepared fresh immediately before use. Spleens were removed from DA and PVG rats and rinsed in phosphate-buffered saline (PBS) to remove excess blood. They were then minced with scissors in PBS and forced through a metal sieve. The resulting cell suspension was washed twice in 0.5% bovine serum albumin (BSA) in PBS by centrifuging at 500 *g* for 10 min.

Red blood cell (RBC) suspensions were prepared by exsanguinating a rat into a syringe containing 200 U of heparin. The blood was washed four times in PBS by centrifuging at 500 *g* for 10 min and removing the buffy coat and upper layer of RBC after each wash. All cell preparations were done at 4°C or on ice.

Antibodies. Immunoadsorbent-purified rabbit $F(ab')_2$ anti-mouse $F(ab')_2$ (RAM) was prepared as described previously (16). RAM was depleted of antibodies cross-reacting

with rat immunoglobulins by passage through a rat $F(ab')_2$ column, and was then designated rat-absorbed RAM. RAM was iodinated using the chloramine T method.

The MN4-91-6 mouse anti-rat class I monoclonal antibody was prepared in the Nuffield Dept. of Surgery, John Radcliffe Hospital, Oxford, England, by fusing spleen cells from a BALB/c mouse immunized with pure DA class I MHC antigens with the myeloma NS-1 (M. R. Newton and J. W. Fabre, unpublished observations). This antibody reacts with a 45,000/12,000 heterodimer, and the target molecule has the expected tissue distribution for class I antigens. It shows a high degree of allospecificity, reacting with DA, PVG $RT1^a$, and PVG $RT1^{u1}$ rats, but not PVG, WAG, or LEW rats.

The F17-23-2 (17) and F16-4-4 (18) antibodies have previously been described in detail. Both are BALB/c spleen/NS-1 hybridomas. F17-23-2 reacts with I/A class II antigens of the DA, LEW, and BN strains, but does not react with the PVG or WAG strains. F16-4-4 reacts with a monomorphic determinant of rat class I antigens.

The MRC OX6 and MRC OX17 antibodies react with monomorphic determinants of the rat I/A and I/E class II homologues, respectively (19). These two cell lines were a kind gift of Dr. A. F. Williams, MRC Immunology Unit, Oxford. All monoclonal antibodies were used as immune ascites partially purified by ion-exchange chromatography.

Immunohistology. Tissue samples (lymph nodes or heart ventricles) for immunohistology were frozen in liquid nitrogen immediately after harvesting. Cryostat sections of 5 μm were cut and air-dried on gelatinized slides, fixed in acetone, and stored at $-20°C$ until used. To stain the sections, the slides were thawed and the appropriate mouse monoclonal antibody was put on the sections at saturating concentrations. This was incubated at room temperature for 30 min and washed twice in Tris-buffered saline. Horseradish peroxidase–coupled rabbit anti-mouse immunoglobulin (Dako, Copenhagen, Denmark), was used at a 1:7 dilution in 20% normal rat serum to block antibodies cross-reacting with rat immunoglobulins. The second incubation was at 37°C for 30 min and the slides were then washed twice. The coloration was developed with diaminobenzidine (Sigma Chemical Co., London) at 6 mg/10 ml containing 3 μl of 100 vol hydrogen peroxide. Sections were counterstained with Harris's hemotoxylin.

Binding Assay. This was performed essentially as described by Morris and Williams (20). All procedures were at 4°C or on ice. Duplicate 25-μl samples of immune ascites to be assayed were transferred to LP3 tubes (Luckham Ltd., Burgess Hill, Sussex). For class II assays, 25 μl of target DA spleen cells at 10^8/ml in 0.5% BSA/PBS was added to each tube, and this was incubated for 1 h on ice. The cells were then washed twice in 0.1% BSA/PBS, and 100 μl of ^{125}I-labeled rat-adsorbed RAM in 0.5% BSA/PBS (~300,000 cpm/tube) was added to the pellet of the second wash. This was resuspended and incubated for 1 h on ice, and the cells were washed twice as above. The pellet of the second wash was resuspended in 0.5 ml of PBS and transferred to fresh LP3 tubes, and the target cell–bound radioactivity was measured in a gamma counter (LKB Produkter, Bromma, Sweden). For class I assays, target cells were RBC at 10^9/ml in 0.5% BSA/PBS and the RAM used was not depleted of rat cross-reacting antibodies.

Absorption Analysis. Initial titrations of immune ascites were performed to choose a dilution that represented conditions of target antigen excess in the assay system. Absorptions were then performed at this dilution, by incubating equal volumes (80 μl) of antibody at the chosen dilution in 0.5% BSA/PBS with tripling dilutions of homogenates or cell suspensions in 0.5% BSA/PBS. Again, all procedures were at 4°C or on ice. Absorbing tissue was removed by centrifugation, and supernatants were assayed in the binding assay as above.

For the absorptions, homogenates were used at starting concentrations as prepared above, i.e., 50% solid tissue, which corresponded to 25–35 mg homogenate protein/ml. Spleen cell suspensions were used at starting concentrations of 5×10^8 nucleated cells/ml.

Results

Basic Studies

In the DA-to-PVG strain combination, cardiac allografts cease to beat on the 6th–9th d after transplantation. Individual survival times in our laboratory for five grafts were 6, 7, 8, 8, and 9 d.

In the initial series of experiments, three allografts and two isografts were removed for immunohistological studies at days 1, 3, 5, and 7 after transplantation. All three allografts at any particular time interval gave virtually identical results, as did both isografts at each time interval. This was followed by quantitative absorptions, which were performed on homogenates of five allografts and seven isografts. All grafts were removed on the 5th d after transplantation and pooled before homogenization.

Class I MHC Antigens

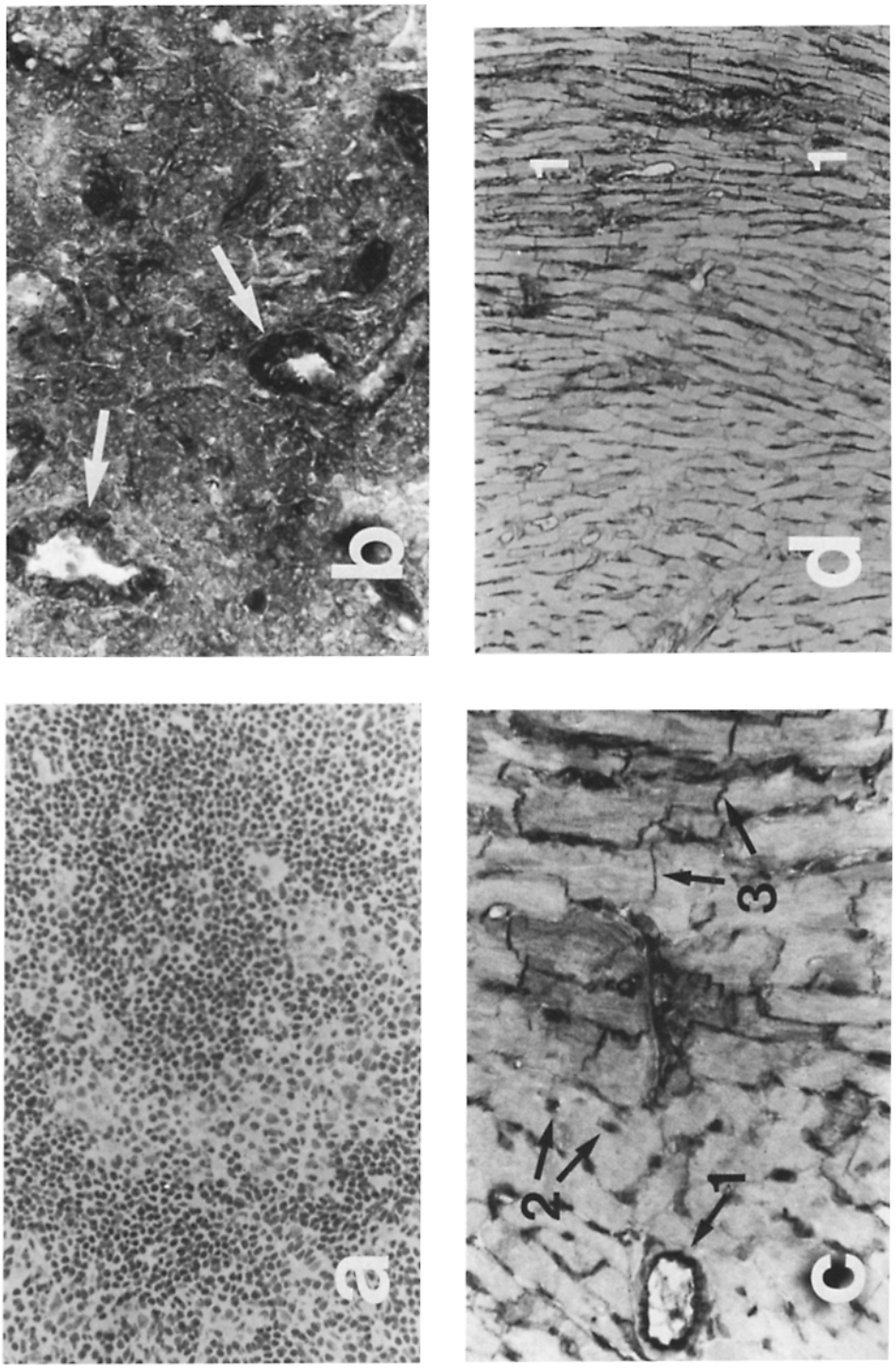
Immunohistological studies. The specificity of the MN4-91-6 antibody for DA is demonstrated in Fig. 1, *a* and *b*. It can be seen that there is strong staining of DA lymph node, but no staining at all of PVG lymph node. Clearly, therefore, in the DA-to-PVG cardiac allografts, all staining will be of cardiac origin, with no contribution from the infiltrating PVG leukocytes. This is obviously critical for meaningful evaluation of the following experiments.

The distribution of class I MHC antigens in the normal DA heart is shown in Fig. 1 *c*. The major staining is seen in interstitial areas and probably mainly represents staining of capillary endothelium, with some staining of interstitial dendritic cells and possibly other leukocytes. The myocardial cell membrane does not stain for class I antigens, except in the region of the intercalated discs. The myocardial membrane probably does express small amounts of class I antigen, and this becomes obvious in the region of the intercalated discs, because of the apposition of the cell membranes. It is also possible, however, that no class I antigen is expressed on the surface of the myocardial cells. Allografts removed 1 d after grafting were not noticeably different from normal DA hearts, either with routine histology or in respect to class I antigen distribution.

By the 3rd d after transplantation, distinct induction of class I antigens could be seen in and surrounding areas of cellular infiltration, as illustrated in Fig. 1 *d*. (Consecutive sections were completely negative with control antibodies.) Although cellular infiltrates were usually associated with class I antigen induction, areas of induction with no obvious cellular infiltrate were visible. There was some staining of the myocardial membrane, but much of the staining was diffusely intracellular.

The picture at the 5th d after transplantation was quite striking, with strong staining for class I antigens throughout the myocardium of the allografts, (Fig. 1, *e* and *f*). Strong staining of the myocardial membranes was obvious. It is particularly worth noting that there was no staining of the interstitial connective tissues, which indicates that most of the class I induction occurs on the myocardial cells.

By day 7 after allografting, the heartbeat was diminished, and sometimes the only activity was surface fibrillation. Histologically, there was much edema and necrosis. Some patches of morphologically distinguishable myocardial cells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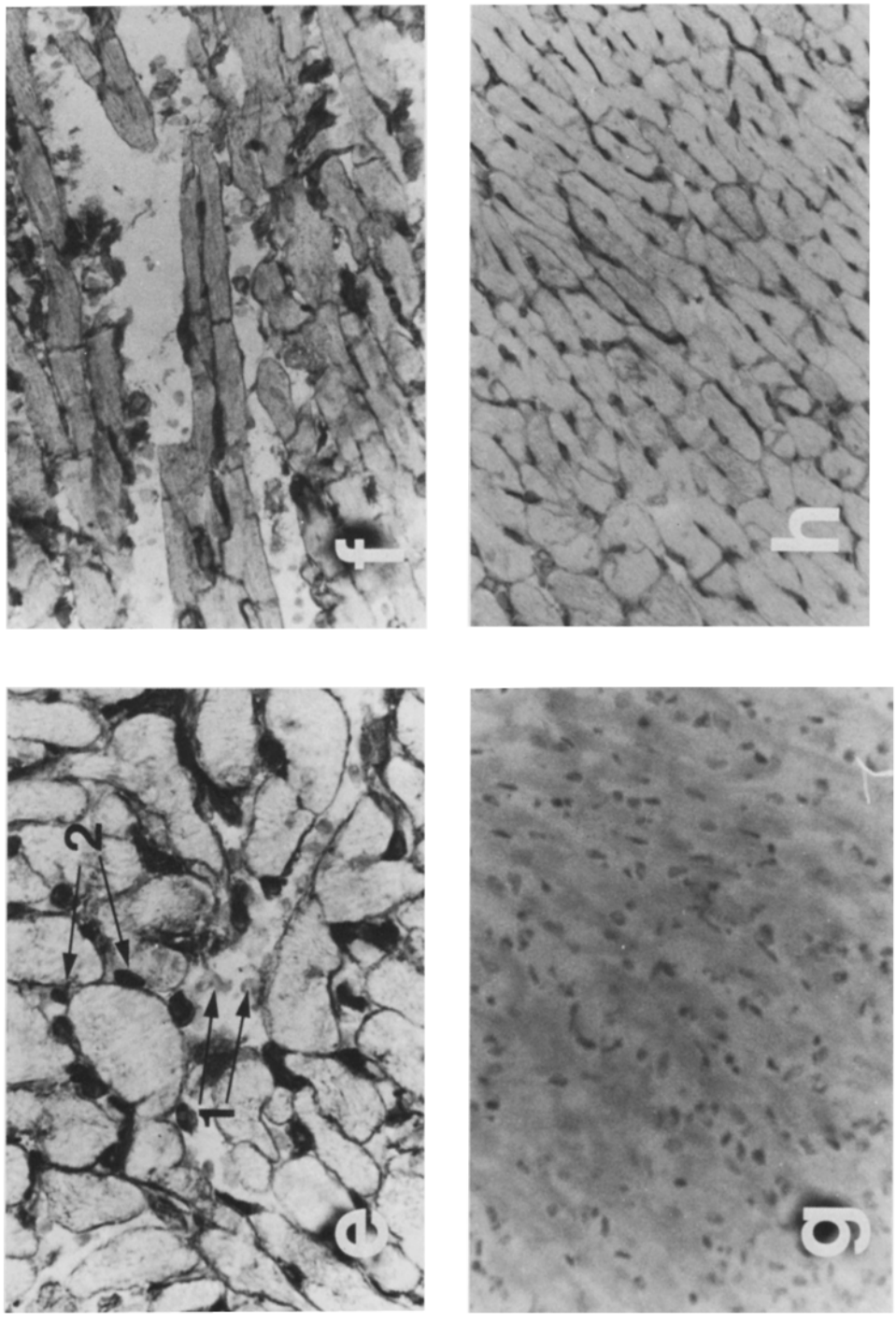


FIGURE 1. Immunohistological studies for class I MHC antigens. Cryostat sections were stained with the MN4-91-6 antibody and peroxidase-labeled anti-mouse immunoglobulin as described in Materials and Methods. (*a*) PVG lymph node. × 250. (*b*) DA lymph node. × 250. Postcapillary venules are indicated by arrows. (*c*) Normal DA heart. × 250. *1*, Arteriole; *2*, capillary endothelium; *3*, intercalated discs. (*d*) DA-to-PVG cardiac allograft, day 3. × 100. *1*, Area of class I antigen induction. Note the clear outline of myocardial membranes, not seen on left of photomicrograph. (*e*) DA-to-PVG cardiac allograft, day 5. × 400. Note strong staining of myocardial cell membrane. *1*, Unstained host infiltrating cells; *2*, probably donor capillaries. (*f*) DA-to-PVG cardiac allograft, day 5. × 250. Note strong staining of myocardial membrane. (*g*) DA-to-PVG cardiac allograft, day 5. × 250. Medium control. (*h*) DA-to-DA cardiac isograft, day 5. × 250. Note weak but definite staining of myocardial membranes.

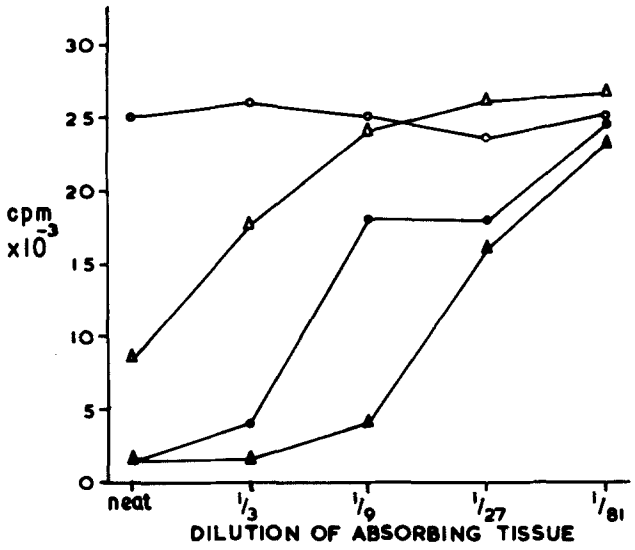


FIGURE 2. Expression of class I MHC antigens in rejecting DA-to-PVG cardiac allografts. Quantitative absorption analyses were performed with the MN4-91-6 antibody and homogenates of normal DA heart (△) and DA-to-PVG cardiac allografts removed at 5 d after transplantation (▲), and DA (○) and PVG (●) spleen cell suspensions. The neat point for spleen cells was 5×10^8 nucleated cells/ml, and for homogenates, 50% solid tissue. Counts per minute refer to ^{125}I-labeled RAM bound in binding assays, using DA RBC as targets.

stained only weakly for class I antigens, perhaps because they were dying cells. Other myocardial cells, however, still stained very strongly for class I antigens.

The DA-to-DA cardiac isografts, included as controls, gave unexpected results. By day 5 it was clear that the myocardial cells were expressing increased levels of class I antigen, as shown by membrane staining with the MN4-91-6 antibody (Fig. 1*h*), but the intensity of staining was much less than with the allografted heart. Increased class I expression was seen throughout the isograft, although it was weaker in some areas than others. With the isografts, it is important to note that the MN4-91-6 antibody reacted with host infiltrating cells, as well as the donor heart, but this did not obscure the myocardial staining.

All of the above immunohistological studies were also performed with the F16-4-4 antibody, which reacts with a monomorphic determinant of class I molecules, in contrast to the MN4-91-6 determinant. Although this antibody stains recipient infiltrating cells in addition to the tissues of the heart graft, the results confirmed the strong staining of myocardial cells for class I antigens, and mirrored the results with MN4-91-6.

Quantitative absorption analyses. The results in Fig. 2 demonstrate that by day 5 after transplantation the allografted DA hearts had increased their level of expression of class I antigens by a factor of 10. The absorption curves with DA and PVG spleen cells illustrate the specificity of the MN4-91-6 antibody for DA.

The results given in Fig. 3 show a threefold increase in class I expression by day 5 in DA-to-DA heart isografts. It should be stressed that the absorption by the DA isograft will include the recipient infiltrating cells, so the threefold

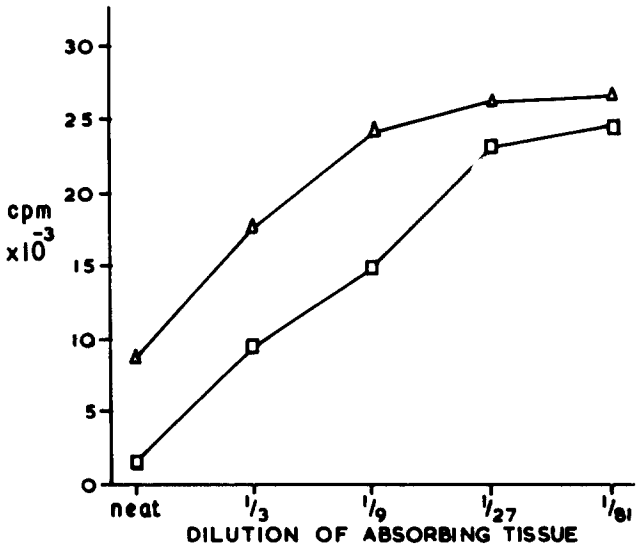


FIGURE 3. Expression of class I MHC antigens on DA-to-DA cardiac isografts. Quantitative absorption analyses were performed with the MN4-91-6 antibody and homogenates of normal DA heart (△) and DA-to-DA cardiac isografts removed 5 d after transplantation (□). The neat point was 50% solid tissue. Counts per minute refer to ^{125}I-labeled RAM bound in binding assays using DA RBC as targets.

increase represents an overestimate of the increased donor expression of class I antigen.

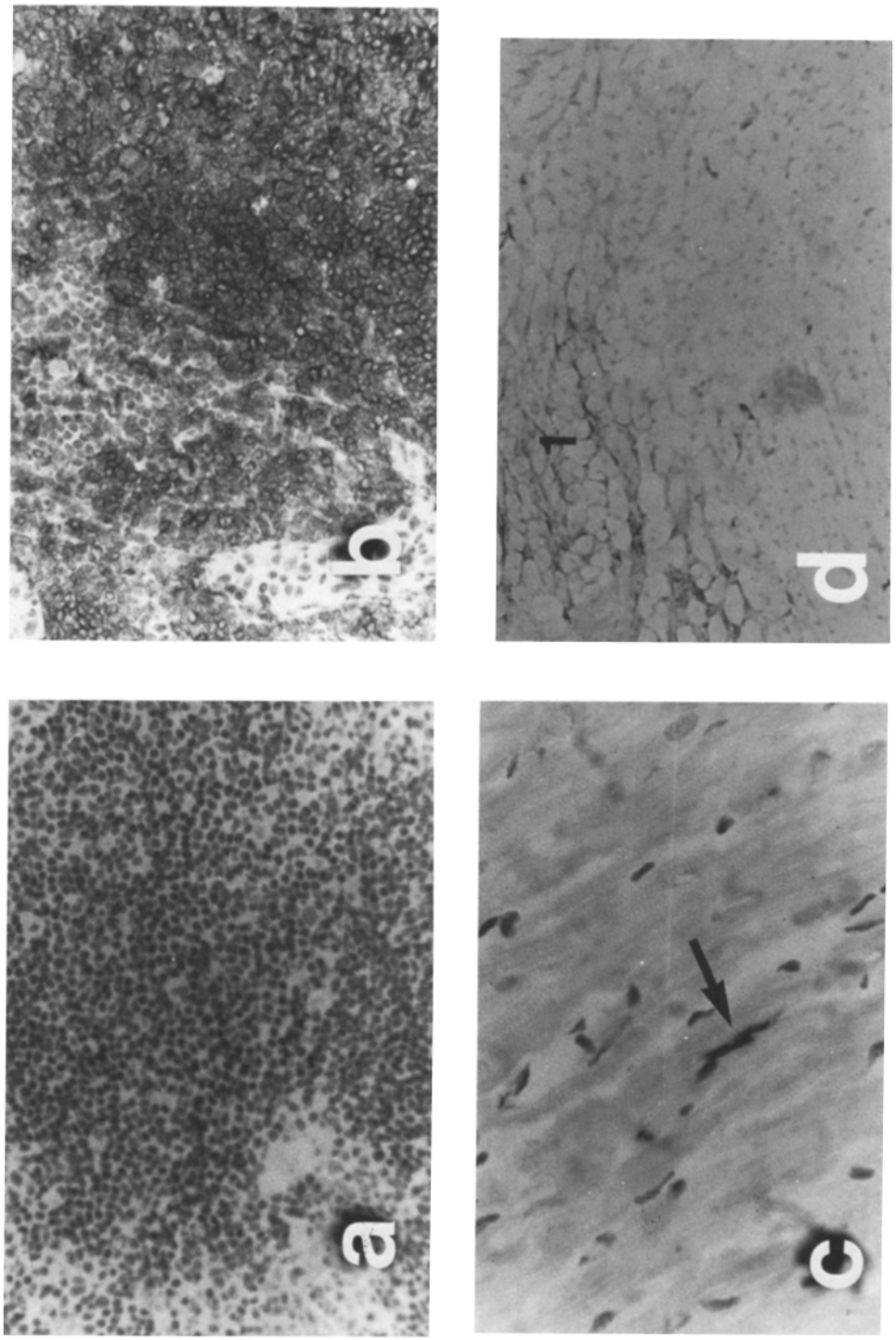
Class II Antigens

Immunohistological studies. The specificity of the F17-23-2 antibody for DA is demonstrated in Fig. 4, *a* and *b*. There is intense staining of the DA, but no staining of the PVG lymph node. As with the MN4-91-6 antibody, this means that F17-23-2 clearly defines antigens of donor origin in the DA-to-PVG cardiac allografts.

It has been demonstrated previously (21) that the only class II–positive structures in normal rat heart are interstitial cells (Fig. 4*c*). Myocardial cells and the endothelium of capillaries and larger vessels are all consistently negative.

After 1 d, DA allografts looked exactly like normal hearts with routine histology and immunohistology using F17-23-2. By day 3, however, as with class I antigens, one could see foci of induction of class II antigens on myocardial cells, usually in association with cellular infiltrates (Fig. 4*d*). Within these areas of induction, there appeared fairly numerous positive interstitial structures, which might represent class II induction on capillaries. However, as shown in Fig. 4*e*, the endothelium of large vessels was consistently class II negative at this stage.

By the 5th d after allotransplantation, there was a strong induction of class II antigens in the myocardium. As can be seen in Fig. 4*f*, the myocardial cell membranes were distinctly and strongly positive. Moreover, as shown in Fig. 4*g*, the endothelium of the large vessels was now also strongly positive for class II antigens. There was some increase in the staining of interstitial structures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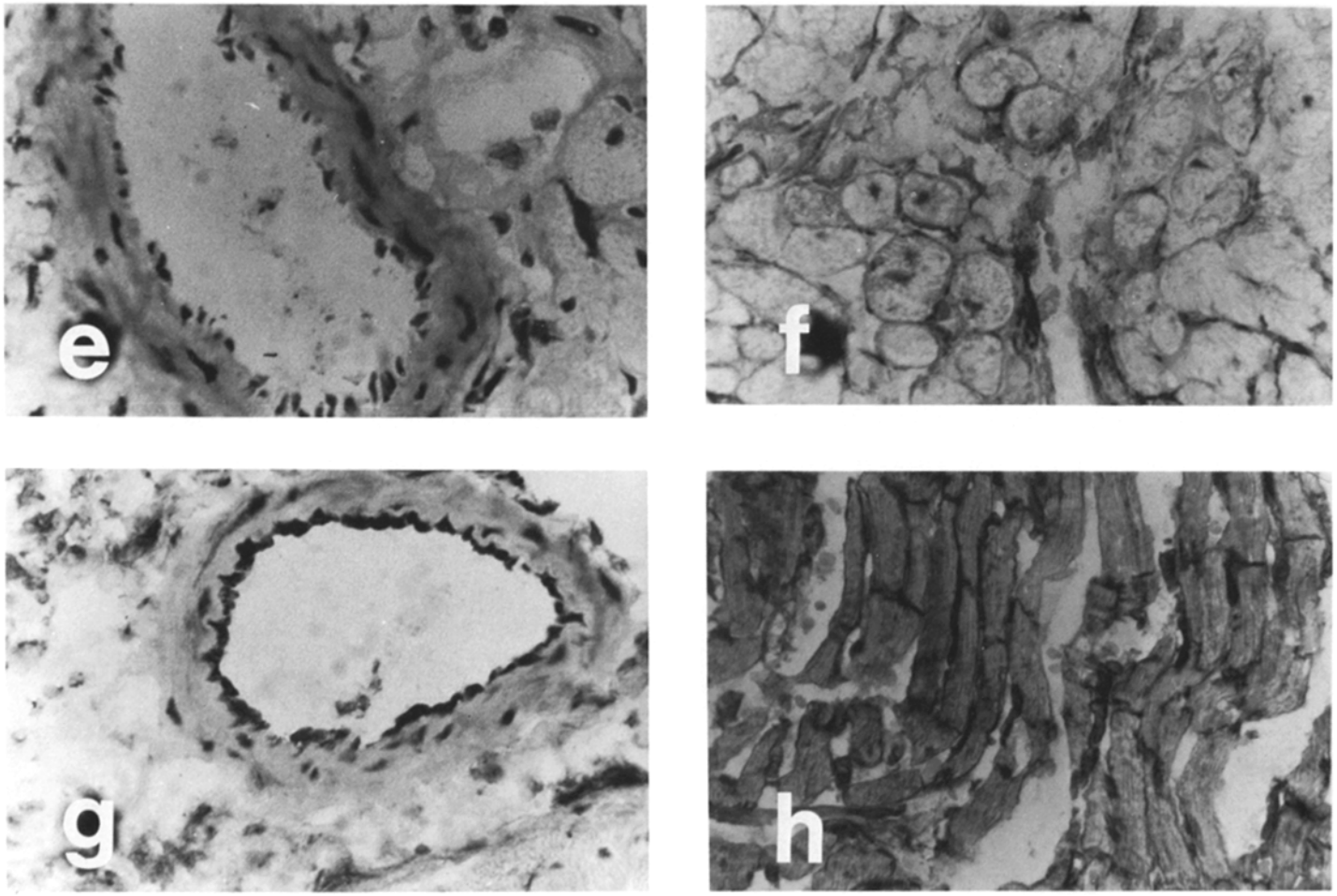

FIGURE 4. Immunohistological studies for class II MHC antigens. Cryostat sections were stained with the F17-23-2 antibody and peroxidase-labeled anti-mouse immunoglobulin, as described in Materials and Methods. (*a*) PVG lymph node. × 250. (*b*) DA lymph node. × 250. (*c*) Normal DA heart. × 400. Arrow indicates a positive interstitial cell. Myocardial cells are negative. (*d*) DA-to-PVG cardiac allograft, day 3. × 100. *1*, Areas of class II MHC antigen induction. Note the clear outline of myocardial membranes, not seen in the other region of the photomicrograph. (*e*) DA-to-PVG cardiac allograft, day 3. × 400. Note the negative endothelium of the arteriole. (*f*) DA-to-PVG cardiac allograft, day 5. × 400. Note the strong staining of myocardial membranes. (*g*) DA-to-PVG cardiac allograft, day 5. × 250. Note strongly positive vascular endothelium. (*h*) DA-to-PVG cardiac allograft, day 7. × 250. Note the very strong induction of class II antigen on the myocardial cells (compare with *c*).

(possibly capillary endothelium), but this was not uniform through the graft. Class II antigens were still being strongly expressed in allografts 7 d after grafting, as shown in Fig. 4*h*. With the DA-to-DA isografts, we could not see any induction of class II antigens.

All of the above immunohistological studies were repeated with the MRC OX6 (directed at the I/A homologue) and MRC OX17 (directed at the I/E homologue) antibodies (19). MRC OX6 and MRC OX17 react with monomorphic determinants of class II antigens and therefore stain recipient infiltrating cells as well as the tissues of the graft. We used these additional antibodies for two reasons. First, we wanted to confirm our results with F17-23-2 using antibodies directed against different determinants on class II molecules. Second, F17-23-2 is directed at a polymorphism of the I/A homologue, and we were interested in studying the I/E homologue as well.

MRC OX6 showed induction of class II antigens on myocardial and vascular endothelial cells, the results being precisely the same as those with F17-23-2 (although staining of the cellular infiltrate by MRC OX6 obscured matters). With MRC OX17, the staining of the cellular infiltrates was as strong with MRC OX6, but staining of the myocardial and vascular endothelial cells, although definite, was perceptibly weaker than with MRC OX6. This might simply reflect differences in the affinity or other properties of these two antibodies, but it could also suggest that induction of I/A class II antigens on myocardial cells was substantially more marked than that of I/E class II antigens.

Quantitative absorption analyses. The F17-23-2 antibody proved unsuitable for absorption analyses over a wide range of dilutions. This was probably because of low affinity and consequent failure to achieve low free-antibody concentrations even with excess antigen in the absorption. This was also the case for the MRC OX17 antibody, but MRC OX6 gave good absorption curves. We therefore proceeded with absorptions using the MRC OX6 antibody, even though it does not distinguish donor class II from recipient class II on infiltrating cells. The results in Fig. 5 show that the content of class II antigen in the allograft had increased substantially by day 5, from nondetectable levels at the time of grafting. Although by immunohistology, much of the class II antigens in the allograft at day 5 appeared to be on myocardial cells, it is not possible to determine the relative contributions of the donor myocardial cells and recipient infiltrating cells to the absorption seen in Fig. 5.

Discussion

The constitutive expression of class I molecules on most nucleated cells might be concerned with the effective presentation of foreign (e.g., viral) antigens to T lymphocytes, particularly cytotoxic T lymphocytes. The level of expression of class I molecules in different tissues varies markedly (22), and, in some cells (e.g., exocrine cells of the pancreas and salivary glands), class I molecules cannot be demonstrated, even with sensitive immunohistological techniques (23). The factors involved in regulating class I antigen expression are not known, but α, β, and γ interferons have been shown to increase the level of expression in many cell types in vitro (9, 10). The physiological role of interferon-induced class I

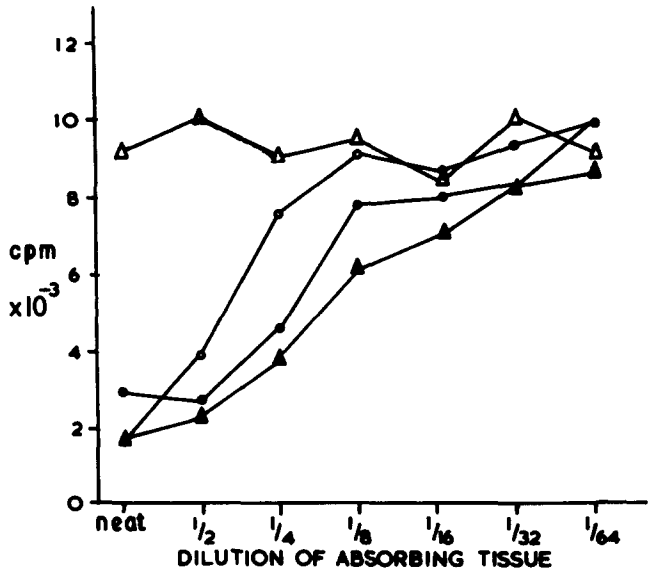

FIGURE 5. Expression of class II (I/A) antigens in rejecting DA-to-PVG cardiac allografts. Quantitative absorption analyses were performed with the MRC OX6 antibody, and homogenates of normal DA heart (△) and DA-to-PVG cardiac allografts removed 5 d after transplantation (▲) and DA (○) and PVG (●) spleen cells. The neat point for spleen cells was 5×10^8 nucleated cells/ml, and for homogenates, 50% solid tissue. Counts per minute refer to ^{125}I-labeled rat-absorbed RAM (see Materials and Methods) bound in binding assays using DA spleen cells as targets.

antigen expression might be to increase the effectiveness of antigen presentation to cytotoxic T lymphocytes in areas of inflammation.

Class II antigens are constitutively expressed on many fewer cell types (e.g., B lymphocytes, dendritic-type cells, and certain epithelia) (24). γ Interferon has been shown to induce class II antigens on various cell types in vitro (9, 10). It is likely that this lymphokine is responsible for the expression of class II antigens on activated macrophages and T lymphocytes (9), on skin and colonic epithelium (11, 12) and kidney during graft-vs.-host disease (13), and on the vascular endothelium (14) and epithelial cells (15) of rejecting skin allografts. It might also be responsible for the patchy expression of class II antigens on some colorectal tumor cells (25). As with class I antigen induction, the physiological role of class II antigen induction might be to improve antigen presentation (to T helper cells) at sites of inflammation. This possibility is greatly strengthened by the recent demonstration (in transfection experiments with mouse fibroblasts) that class II antigen expression is by itself sufficient for effective antigen presentation by a cell (26).

It can be argued that MHC antigen induction at sites of inflammation might represent a favorable adaptive response, improving host defenses and therefore favoring the individual's survival. However, in the unnatural situation of a tissue graft residing in a foreign host, this response is very likely to evoke more effective rejection responses and lead to the demise of the graft (and sometimes its host).

In the particular situation examined in our experiments, the 10-fold increase

in donor class I antigen expression by the rejecting heart, probably mainly on myocardial cells, would probably increase the susceptibility of the myocardial cells to class I–specific cytotoxic T cells, especially since myocardial cells normally express few, if any, class I molecules. This increased level of expression might also favor the maturation of class I–specific cytotoxic T cell precursors.

The expression of class II molecules on both myocardial and endothelial cells could be of particular importance, because of the major role played by these molecules in T helper lymphocyte activation. The large-scale induction of class II antigens in the rejecting heart might greatly augment activation of T helper cells and thereby provide a continued source of T helper cell–derived γ interferon to maintain class II (and class I) antigen induction in the myocardial and endothelial cells. In this way, an expanding and self-regenerating cycle could be initiated that would terminate with the destruction of the graft. The induced class II antigens could also serve as new targets on endothelial and myocardial cells for class II–specific cytotoxic T cells, and, as indicated above, might enable the myocardial cells to present class I and minor alloantigens to T helper cells.

The induction of class I MHC antigens on the DA-to-DA cardiac isografts was unexpected. It is possible that activation of graft macrophages and dendritic cells, and perhaps the infiltration of the graft with recipient macrophages as a result of the surgical trauma and ischemia at the time of transplantation, are responsible. The activated macrophages might secrete α and β interferons, which are potent inducers of class I but not class II MHC antigens (10). The induction of class II antigen probably requires infiltration of the graft with activated T lymphocytes secreting γ interferon, and this would occur only in the allograft situation.

In conclusion, we would like to stress that the strong induction of class I and class II MHC antigens during the rejection process, initiated at sites of perivascular infiltration, presumably by the release of lymphokines, might provide a strong driving force for rejection responses, and could also increase the graft's vulnerability to the rejection process by providing a more effective target for the host's immune system.

Summary

DA ($RT1^a$) hearts were transplanted into PVG ($RT1^c$) or DA recipients, excised on days 1, 3, 5, or 7 after grafting, and examined by immunohistological techniques and quantitative absorption analyses, using allospecific mouse anti-rat class I and class II major histocompatibility complex (MHC) monoclonal antibodies. Cryostat sections stained by the peroxidase technique demonstrated that, in the normal heart, class I antigens were largely restricted to vascular endothelium and interstitial cells, with no observable staining of the myocardial cells except at the intercalated discs. Class II antigens were found only on occasional interstitial dendritic cells. The picture at day 1 after transplantation was not noticeably different. By day 3, however, there was clear patchy induction of both class I and class II antigens on the myocardial cells, usually in the region of cellular infiltrates. By day 5, class I antigens had been strongly induced throughout the graft, with the myocardial cells being very strongly positive. Class II antigens were also uniformly expressed on myocardial cells at day 5, and at this stage the

vascular endothelium was also strongly positive. Quantitative absorption analyses showed a 10-fold increase in class I antigen content in cardiac allografts at day 5 after transplantation when compared with normal DA heart. DA heart isografts showed no increase in class II antigens, but it was interesting that, by 5 d after grafting, there appeared to be some expression of class I antigens on the myocardial cells. Quantitative absorptions showed a threefold increase in class I antigens on 5-d isografts when compared with normal DA heart.

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