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Altered distribution of MHC class II antigens on enterocytes during acute small bowel allograft rejection in rats

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Abstract. Class II major histocompatibility complex (MHC) antigen induction was investigated on enterocytes of heterotopic rat small bowel allografts in the Lewis-Brown Norway strain combination and on isografts in the Lewis-Lewis strain combination. Ia antigens were detected with monoclonal antibodies using an immunoperoxidase technique. Generally, MHC class II antigens were not exhibited in the isografted group, with the exception of two long-term isografts that presented the same pattern as normal small bowel. In these cases, Ia was expressed in a patchy distribution predominantly in the villi, and only very few enterocytes stained positive in Lieberkühn's crypts. Allografted rats showed a typical pattern of Ia expression on the enterocytes during the rejection course. The initial expression was confined to the crypts, indicating a very early stage of rejection when compared to histological findings. More advanced stages of rejection were accompanied by increasing Ia biosynthesis in the crypts and Ia expression by the epithelium lining the villi. Cyclosporin (CyA) was not able to fully inhibit MHC class II antigen expression; however, the appearance of Ia was delayed.

Key words: Small bowel transplantation, rat – Rejection, small bowel transplantation, rat – Class II antigens, small bowel transplantation, in rats

Major histocompatibility complex (MHC) class II antigens (Ia) are polymorphic, membrane-bound glycoproteins. They play an integral role in the regulation of immune responses and are induced by soluble mediators [22]. These antigens were first demonstrated on a variety of lymphoreticular cells including B lymphocytes, activated T lymphocytes, monocytes, macrophages, and dendritic cells [26]. It soon became evident that epithelial cells were also capable of synthesizing class II antigens as a physiological phenomenon. Ia antigens were detected on epithelial cells of the thymus [4], gut, kidney, bronchi [18], mammary gland (particularly during lactation) [13], and on the vascular endothelium in men [19]. Class II antigens can also be expressed in disease states [15] in cells generally considered Ia-negative, something which has been demonstrated in autoimmune diseases [1, 14]. Finally, Ia antigen expression was found on epithelial cells of various allografts, and it became obvious that the degree of class II antigen expression was altered during the rejection process [11, 24].

In this study we were able to show that class II antigens were expressed by the majority of enterocytes in rejected small bowel allografts in the rat. The impact of different phases of rejection on the expression of Ia antigens was investigated. Finally, the influence of cyclosporin A (CyA) on the expression of these antigens was evaluated. The monitoring of the expression of Ia by enterocytes of small bowel allografts might be helpful in establishing rejection diagnosis in the early postoperative phase.

Materials and methods

Accessory small bowel transplantation using microsurgical techniques was performed in rats. The entire small bowel from Treitz's ligament to the ileocecal valve was harvested from inbred male Lewis (LEW) rats weighing between 200 and 270 g and transplanted into male LEW or Brown-Norway (BN) rats (Zentralinstitut für Versuchstierzucht, Hannover, FRG) of equal size. The LEW→BN strain combination represents a low responder combination. After in situ flushing of the graft with 5 cc of cold saline solution, graft vessels (portal vein and superior mesenteric artery) were anastomosed to the infrarenal aorta and vena cava, respectively. All grafts were placed in a heterotopic position. The oral end of the graft was closed and the distal end joined in a Roux-en-Y fashion to the distal ileum of the recipient [8]. Postoperatively, all animals were given water and a standard rat chow diet ad libitum.

Three groups were studied. In group 1 ($n = 18$), LEW rats received isografts and no immunosuppressive treatment. Group 2 ($n = 10$) consisted of BN recipients of LEW grafts treated with CyA orally (10 mg/kg body weight per day). Group 3 ($n = 10$) included BN recipients that received grafts from LEW donors and no immunosuppression.

In group 1, five animals were sacrificed on day 5 (subgroup 1a) and another five around day 10 (subgroup 1b), while the remaining eight rats in this group were observed for as long as 25–46 days (subgroup 1c). This last subgroup was established in order to study long-term changes in accessory small bowel isografts. Half of the animals

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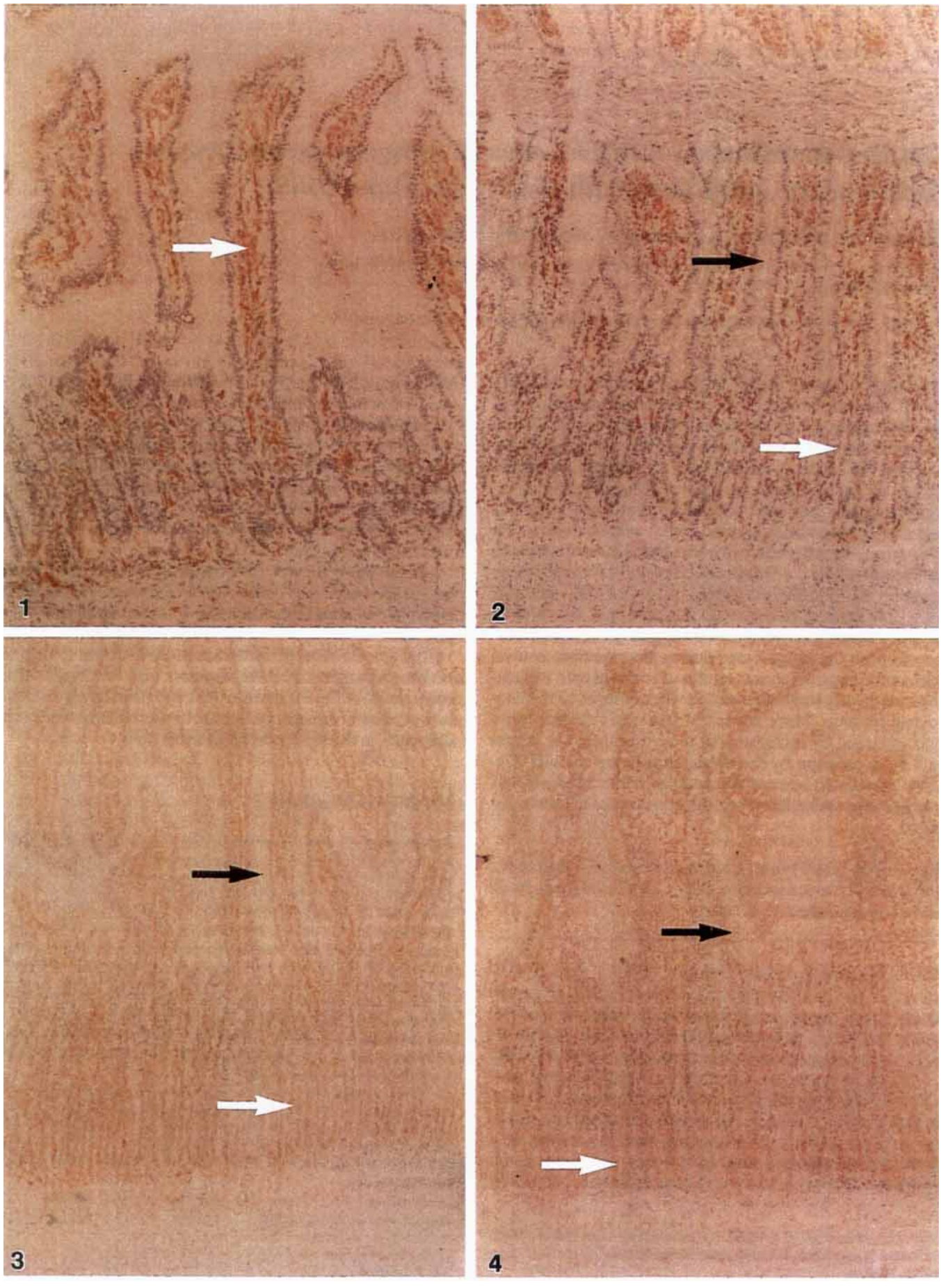
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in groups 2 and 3 were sacrificed 5 days after transplantation (subgroups 2a, 3a), and the other half between days 8 and 10 (subgroups 2b, 3b).

Four monoclonal mouse anti-rat Ia antibodies – MRC OX-3, MRC OX-6, MRC OX-17 [9], and F 17-23-2 [11] – were used to detect Ia antibodies in frozen sections. F 17-23-2 reacts with a polymorphic determinant of the rat MHC class II antigen homologue of murine I/A. It reacts with LEW and BN strains. MRC OX-6 and MRC OX-17 monoclonal antibodies react with monomorphic determinants of the rat I/A and I/E class II homologues, respectively. MRC OX-3 reacts with a polymorphic determinant of the rat MHC class II antigens; it recognizes Ia of LEW rats but not of BN rats.

Three circumferential cross sections were cut from corresponding areas of each graft and immediately frozen in liquid nitrogen. Cryosections 4–5 µm thick were air-dried, fixed in acetone for 20 min at – 20°C, in chloroform for 10 min at room temperature, and rehydrated in phosphate-buffered saline (PBS). Subsequently, endogenous peroxidase was incubated with a solution consisting of glucose (10 nM), glucose-oxidase (IU/ml), and sodium acetate (1 m*M*, Sigma). The sections were then incubated with the respective mouse monoclonal antibody at saturating concentrations for 1 h. The sections were subsequently rinsed twice in PBS and developed with a sheep anti-mouse IgG peroxidase conjugate (DAKO). After three washes in PBS, the enzyme reaction was developed in a freshly made solution of diaminobenzidine (0.5 ng/ml, Sigma) and 0.01% H_2O_2 (Merck) for 5 min at room temperature. All sections were counterstained with hematoxylin, dehydrated, and cleared in xylene and mannitol.

In addition, five circumferential cross sections were fixed in buffered formaline and embedded in paraffin. Five micrometer thick sections were then stained with hematoxylin and eosin for histological classification of the severity of rejection [23].

All antibody-stained slides were analyzed by an investigator who was blinded to the various experimental groups. The Ia expression on the enterocytes lining the villi, as well as in Lieberkühn's crypts, was graded on a semiquantitative scale (– , + , + + , + + +). These findings were then compared with the histological classification of the rejection process.

In addition to the grafts, the normal small bowel of untreated LEW rats was examined and served as a control.

Results

MHC class II antigen expression in the normal small bowel (Fig. 1)

Ia was found in a patchy distribution predominantly in the villi. Only very few enterocytes in Lieberkühn's crypts were positive.

Fig. 1. Normal small bowel. Patchy distribution of MHC class II positive enterocytes predominantly in the villi. Note brown-stained Ia-positive stromal cells *(arrow)*. OX-6, diaminobenzidine *(DAB)*, ×300

Fig. 2. Small bowel isograft. Enterocytes in the villi *(black arrow)* as well as in Lieberkühn's crypts *(white arrow)* are negative for Ia. Only stromal cells stain positive. OX-6, *DAB*, ×300

Fig. 3. Stage 1 (small bowel allograft, histologically demonstrating an early phase of rejection). Lieberkühn's crypts *(white arrow)* are weakly stained with OX-6, while the villi *(black arrow)* remain negative. OX-6, *DAB*, ×300

Fig. 4. Stage 2 (small bowel allograft, histologically demonstrating a moderate rejection). Lieberkühn's crypts *(white arrow)* are stongly Ia-positive. In this stage the enterocytes of the villi *(black arrow)* also stain weakly with OX-6. OX-6, *DAB*, ×300

MHC class II antigen expression in heterotopic small bowel isografts (Fig. 2)

Enterocytes in subgroups 1a (5 days) and 1b (10 days) did not express Ia. The same was true for the majority of animals in subgroup 1c (25–46 days). In two grafts in this group, however, that were in situ for 38 and 46 days, the same pattern of MHC class II antigen expression was seen as in the normal small bowel.

MHC class II antigen expression in heterotopic small bowel allografts (Table 1)

As shown in Table 1, three typical patterns of Ia expression were seen in allografted animals. They were referred to as stages 1, 2, and 3. It became evident that the immunological findings correlated with the histological grade of the rejection process.

Stage 1 (Fig. 3). Enterocytes of Lieberkühn's crypts showed a weak Ia expression, whereas the enterocytes lining the villi remained negative. This pattern was seen in four animals in group 2a (LEW→BN, 5 days, CyA). These grafts histologically showed an early phase of rejection. The basal layers of the lamina propria and the submucosa were weakly infiltrated by eosinophils and a few mononuclear leukocytes. A mononuclear infiltrate was seen around the myenteric ganglia. One animal in group 2a (LEW→BN, 5 days, CyA), which did not show any histological signs of rejection, however, exhibited the same pattern of MHC class II antigen expression as the isografts.

Stage 2 (Fig. 4). Enterocytes of Lieberkühn's crypts were strongly Ia-positive and epithelial cells lining the villi were weakly stained. This pattern of MHC class II antigen expression was exhibited by all animals in group 2b (LEW→BN, 10 days, CyA) and group 3a (LEW→BN, 5 days, no immunosuppression). This situation corresponds to a moderate rejection with dense infiltration of the lamina propria and of the submucosa by mono- and polynuclear leukocytes. A few lymphocytes were also scattered around the myenteric ganglia.

Stage 3. Enterocytes of the crypts as well as of the epithelium lining the villi were strongly Ia-positive. All animals in group 3b (LEW→BN, 10 days, no immunosuppression) belong to this stage. This pattern of MHC class II antigen expression histologically represents a severe rejection. Slides showed heavy infiltration of the lamina propria and the submucosa by mono- and polynuclear leukocytes. The enterocytes were cubed in shape, goblet cells had disappeared, and the villi were flattened. In addition, the muscular wall was infiltrated by lymphocytes and neutrophils. Two grafts were histologically completely destroyed, and islets of preserved epithelial cells were strongly Ia-positive.

All epithelial cells expressing Ia showed an equally distributed staining with anti I/A and anti I/E antibodies.

OX-3, an antibody able to recognize class II determinants of LEW but not of BN, stained Ia-positive enterocytes, indicating that these antigens had not been passively absorbed from infiltrating Ia-positive host cells.

Table 1. Stages of I a expression in small bowel allografts

Stage	Group	Histology	I a expression	
			Crypts	Villi
1	2a[a]	Beginning rejection	+/+ +	−
2	2b, 3a	Moderate rejection	+ +/+ + +	+
3	3b	Severe rejection	+ + +	+ +

[a] Four of five grafts in group 2a. One graft in this group, however, which histologically did not show any signs of rejection, exhibited the same pattern of I a as isografts

−, +, + +, + + +, relative degrees of immunofluorescence staining. Group 2a: allografts, 5 days, CyA; group 2b: allografts, 10 days, CyA; group 3a: allografts, 5 days, no CyA; group 3b: allografts, 10 days. no CyA

Discussion

De novo expression of class II antigens by nonlymphoid cells has been seen in several organ grafts: on keratinocytes and endothelial cells in skin grafts [7], on myocardial and endothelial cells in cardiac grafts [17], on tubular epithelial cells in kidney grafts [6], on the bronchoepithelium in lung allografts [21], and on duct epithelial and acinar cells of pancreas grafts [24].

Weak I a expression on normal enterocytes was first described by Cerf-Bensussan et al. [5]. We also found a patchy distribution of I a on villi of normal small bowel. This was not seen in heterotopic small bowel isografts, probably due to a lack of stimulation by bypassing antigens. In this study we were able to demonstrate that rejection of small bowel allografts was invariably accompanied by the induction of MHC class II antigens in enterocytes. Intensity and pattern of I a expression were well correlated with the severity of rejection according to histological findings. Therefore, a classification into three stages was performed, with stage 1 representing the beginning of rejection. All enterocytes in Lieberkühn's crypts expressed low or intermediate levels of MHC class II antigens. Stage 2 is defined by intermediate or strong staining in the crypts in association with weakly stained enterocytes lining the villi. Stage 3 is defined by strong staining of the crypts in association with intermediate staining of the villi. Stage 2 represents ongoing rejection and stage 3 a late phase of rejection.

An explanation for these different patterns remains speculative. As the expression of I a antigens at the beginning of the rejection process occurs essentially in the crypts, it might be argued that the various immunological patterns are caused by the physiological migration of surface cells from the crypts towards the villi. On the other hand, I a positivity in the villi could primarily be due to de novo antigen expression.

Enterocytes of long-term heterotopic isografts may also synthesize I a, as seen in two cases in group 1c. In those heterotopic grafts that, defunctionalized, represent a blind loop, bacteria may accumulate [10]. Indeed, there was increased migration of inflammatory cells into the lamina propria, something which, again, may trigger MHC class II antigen expression by the epithelium. In contrast to I a expression in allografts, the staining in isografts was found to be equally intensive in Lieberkühn's crypts as well as in the villi. In addition, the distribution of I a-positive groups of enterocytes was patchy in isografts in contrast to allografts during rejection, when I a was homogeneously distributed.

The mechanism underlying the regulation of class II antigen expression is not yet fully understood. Lymphokines released by activated T lymphocytes and macrophages [2], such as interferon gamma, are thought to be responsible for triggering MHC class II expression. This has already been shown in vitro for endothelial cells and macrophages and in vivo for epithelial cells and dendritic cells of various origin. Other lymphokines such as interleukin 4 (IL 4), however, may also be mediators for inducing these antigens. It has been shown that IL 4 has the capacity to enhance MHC class II expression on B lymphocytes in a murine animal model [25].

I a antigen expression by the gut epithelium of rejected allografts may be important in two ways. First, the enhanced antigenicity may be detrimental to the allograft. Indeed, incompatibilities between donor and recipient class II antigens represent a powerful stimulation of the rejection process [27]. Second, the activation of helper T cells by I a-positive enterocytes, analogous to a mechanism already shown for thyreocytes and endothelium [12], as well as the possibly increased susceptibility to cytotoxic T cells [20], might play an important role in the induction of host-versus-graft reaction. Hence, the strong expression of I a by enterocytes may be one of the reasons why small bowel allografting still poses an insurmountable immunological problem. This view is further supported by the fact that at the dosage given, CyA is able to delay but not prevent rejection. It has been shown that very high levels of CyA result in a modest decrease in normal MHC product expression, although this is not true for the usual blood levels of CyA [2].

The observation of altered MHC class II expression during allograft rejection has not led to the general acceptance that I a expression would be a useful clinical marker for rejection monitoring. It is maintained that a variety of stimuli, including infection and contact irritants, are known to induce class II antigen expression on enterocytes. Nevertheless, our findings might support the establishment of a rejection diagnosis in the early postoperative period, when the graft is still exempted from enteral function and nonallograft stimuli are negligible. As has already been reported, mucosal biopsies alone are not sufficient for diagnosis of rejection since most immunological reactions take place in the submucosal and muscular layer [16, 23]. Furthermore, these mononuclear cell infiltrates are not homogeneously distributed within the graft. Both findings require multiple and deep biopsies with a substantial risk of perforating the bowel wall. In contrast, I a expression can be demonstrated on superficial biopsies or exfoliation cytologies, which can easily be obtained. Moreover, I a is already expressed in an early phase of rejection when function of the allograft is not yet impaired [3]. An early diagnosis of rejection based on the detection of I a on enterocytes might allow intensification of immunosuppression at an early stage of host-versus-graft rejection, which should prevent further deterioration of graft function.

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