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Rainer Lück
Jürgen Klempnauer
Birte Steiniger

Genetic requirements for the development of the GVH reaction following small-bowel transplantation

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R. Lück (✉) · J. Klempnauer
Klinik für Abdominal- und Transplantationschirurgie,
Medizinische Hochschule Hannover,
Konstanty-Gutschow-Strasse 8,
D-30625 Hannover,
Germany

B. Steiniger
Abteilung V, Zentrum Anatomie,
Medizinische Hochschule Hannover,
D-30625 Hannover, Germany

Abstract The genetic requirements for the development of graft-versus-host (GVH) disease have been investigated in a model of semiallogenic, heterotopic small-bowel transplantation in the rat. Following semiallogenic MHC-incompatible small-bowel transplantation, all graft recipients showed characteristic signs of GVH disease and died within 14 days. On autopsy the transplanted bowel was normal, while the recipient's bowel was dilated and distended with gas. Histology showed a generalized cell infiltration of the connective tissue with macrophages and lymphocytes. After semiallogenic, RT1.A-incompatible, small-bowel transplantation, the graft recipients developed mild and temporary symptoms of GVH disease between days 25 and 40. Only two of the six animals died, while the remaining animals survived the observation period. Small-bowel transplantation across an isolated RT1.C barrier was unable to induce GVH reaction. These results indicate that the development of GVH disease after small-bowel transplantation is controlled genetically by the MHC. Class II MHC incompatibility is necessary for the induction of an acute and lethal GVH reaction.

Key words GVH disease, small bowel, rat · Small bowel, GVH disease, rat · MHC, small bowel, rat

Introduction

In contrast to other vascularized organs, small-bowel grafts are not only prone to graft rejection, but may also induce graft-versus-host (GVH) reactions [10]. These GVH reactions are mediated by cotransplanted lymphoid tissue within the small-bowel graft. It is well established that a histocompatibility barrier of certain transplantation antigens is necessary for the development of GVH disease [1]. However, immunogenetic data about the genetic requirements for the occurrence of GVH disease after small-bowel transplantation are still not available.

The most important transplantation antigens of all mammas are encoded within the major histocompatibility complex (MHC). The MHC of the rat, the RT1 system, is homologous to the MHC of the mouse and of humans and consists of several closely linked gene regions. Based on differences in the structure, tissue distribution, and function, class I and class II antigens can be distinguished within the MHC. The RT1.A region of a rat MHC encodes the classical class I antigens that are equivalent to the human HLA A, B, C system. The rat RT1.B/D region codes for class II MHC antigens, which corresponds to the human HLA DR, DP, and DQ regions. In the rat, the RT1.C region represents a further class I gene locus that differs in many respects from the RT1.A region. These antigens resemble the mouse H-2Qa antigens, and so far no equivalent in the human MHC has been characterized [2, 5].

The genetic requirements for the development of a GVH reaction have been investigated in a model of semiallogenic, heterotopic small-bowel transplantation in the rat. Using certain rat strains and their recombinants, it is possible to determine the impact of individual MHC antigens on the development of GVH reactions [7]. The latter were investigated after semiallogenic transplantation of

Table 1 Genetic characterization of the rat strains studied

Strain		RT1			Genetic background
		A	B/D	C	
LEW.1A	a	a	a	a	LEW
LEW.1U	u	u	u	u	LEW
LEW.1R3	r3 = ar2	a	a	u	LEW
LEW.1R6	r6 = wr2	u	a	a	LEW
LEW.1R4	r4 = wr1	u	u	a	LEW

Table 2 Differentiation of HVG and GVH reactions with F1 hybrid rats. (*P1* and *P2* Parental rat strains (depending on the incompatibility used), $F_1(P_1{*}P_2)$ P_1 male, P_2 female)

Allogenic transplantation		
	$P_i \longrightarrow$	P_2
	HVG and GVH reaction	
Semiallogenic transplantation		
	$F_i(P_i{*}P_2) \rightarrow P_2$	$P_1 \rightarrow F_1(P_1{*}P_2)$
	HVG reaction	GVH reaction

small-bowel grafts of parental donors to F1 hybrid recipients in which, for genetic reasons, only GVH reactions could occur. We have investigated the impact of total MHC, as well as isolated class I and class II MHC, incompatibilities on the development of GVH reactions after heterotopic transplantation of the entire small bowel.

Materials and methods

Animals

The parenteral and F1 hybrid rat strains used in the study were bred at the Zentrales Tierlabor der Medizinischen Hochschule, Hannover, and the Zentralinstitut für Versuchstierzucht, Hannover, Germany. The RT1 haplotypes and the genetic background of the strains are listed in Table 1. The intra-MHC recombinant rat strains LEW.1R3, LEW.1R4, and LEW.1R6 were derived by segregating hybrids of the strains LEW.1A and LEW.1U. In appropriate combinations, these strains differ either in their entire MHC or in the RT1.A, RT1.B/D, or RT1.C region only. The impact of these genetic differences on the development of GVH reactions was investigated by the transplantation of parental small bowel into F1 hybrid recipients. The F1 hybrid rats were bred using the appropriate pair of parental strains (Table 2). At the time of transplantation, donors and recipients were about 3 months of age and weighed 200–300 g. All animals were kept under conventional conditions in fully climatized rooms with a constant day and night rhythm. They had free access to standard pellet food and water.

Technique of heterotopic small bowel transplantation

All donors were fasted overnight before surgery. For graft removal they were anesthetized i.m. with a dose of 0.2 mg/kg fentanyl and 10 mg/kg fluanison. The whole small bowel of the donor was mobilized from the ligament of Treitz to the cecum. The arterial blood supply was provided by the superior mesenteric artery with an adjacent segment of the aorta. The venous outflow was routed through the portal vein after careful dissection of the pancreas from the superior mesenteric vein. The lumen of the bowel was rinsed free with physiological saline without the addition of antibiotics. The oral end of the small-bowel graft was closed by ligature. The graft was perfused via the superior mesenteric artery with 4 °C cold heparinized (50 IU/ml) physiological saline. The graft was then stored in cold saline. The cold ischemia time never exceeded 30 min.

Without specific pretreatment of the recipient, small-bowel grafts were implanted under i.p. ketamine anesthesia at a dose of 80–120 mg/kg. The arterial blood supply of the graft was restored with an end-to-side anastomosis between the aortic segment of the graft and the recipient's infrarenal aorta. The venous outflow was routed through an end-to-side anastomosis between the donor portal vein and the recipient infrarenal caval vein. The aboral end of the small-bowel graft was anastomosed end-to-side to the last loop of the recipient's ileum. Immediately after transplantation the animals had free access to water; food was added after 48 h. No antibiotics or other drugs were used during or after transplantation.

Monitoring

The animals were examined daily for their clinical appearance, i.e. for the development of symptoms suggesting GVH disease [16], and for their body weight. Autopsy and histological examination were performed immediately after death or when sacrificed at the end of a 100-day observation period.

Histology

For histological and immunohistological evaluation, the transplanted small bowel and the following recipient organs were removed: small bowel, pancreas, heart, lung, liver, kidney, spleen, mesenteric lymph nodes, tongue, esophagus, and ear skin. Representative pieces were cut and either fixed in Bouin's solution for paraffin embedding or immediately frozen and stored in liquid nitrogen for immunohistology. Paraffin sections were stained with hematoxylin-eosin. For the immunohistological demonstration of class II MHC antigens, 10-µm frozen sections of the tongue, esophagus, and ear skin were cut and stained with monoclonal antibody 0×6-detecting products of the I-A subregion equivalent in rats. Excessive background staining of squamous epithelia required modification of our previously published indirect peroxidase technique (demonstration of class II in dendritic cells, [14]). The secondary peroxidase-conjugated antimouse immunoglobulin was diluted in phosphate-buffered saline containing 0.35 M NaCl, 10% bovine serum albumin, and 10% inactivated normal rat serum. The color reaction was developed with diaminobenzidine. The normal distribution of class II MHC antigens in rat organs has been previously described in detail [14].

Table 3 Survival times following semiallogenic (P→F1) heterotopic small-bowel transplantation

Incompatibility	Survival times (days)	Median survival time (days)
MHC	11, 11, 11, 12, 13, 14	11.5
RT1.A	30, 40, $>100\times4$	>100
RT1.B/D	11, 12, 15, 16, 19, 20	15.5
RT1.C	$>100\times6$	>100

Statistical evaluation

The survival times of the individual groups were subjected to the Kruskal-Wallis test for nonparametric, one-way analysis of variance. In cases of significance, the Wilcoxon U-test was performed. The level of significance was set to $P > 0.01$. There were statistically significant differences in graft survival between these groups. The survival times after RT1.A and RT1.C incompatible bower transplantation were longer than in the case of RT1.B/D and MHC disparity. No differences were noted between the groups with MHC and RT.1B/D or RT1.A and RT1.C incompatibility.

Results

Isogenic controls

Within 10 days after isogenic small-bowel transplantation, the graft recipients fully recovered from the sequelae of the operation and could no longer be distinguished from normal, untreated, age-matched rats. Their growth rate and behavior were completely normal. At autopsy on post-transplant day 100, the heterotopically grafted small bowel was slightly shrunken, but histologically normal. No abnormalities were found in the recipient organs either.

MHC incompatibility

Following transplantation of the MHC-incompatible, semiallogenic small bowels, the graft recipients developed an acute and lethal graft-versus-host disease (GVHD). The animals showed all the typical signs of GVHD, such as a distended abdomen, an erythema, and ulcerating dermatitis that mainly affected the mucosa of the eye, mouth, and urogenital tract. The animals progressively lost weight, were no longer physically active, and became blind. Within 14 days after small-bowel grafting, all of the recipients died in a state of complete emaciation. Alopecia or hyperkeratosis were not noted. Diarrhea occasionally occurred in moribund animals. At autopsy the transplanted small bowel was found unchanged, while the cotransplanted mesenteric lymph nodes were enlarged. The recipient's small bowel was dilated and filled with gas. In most cases, intraluminal intestinal or urogenital bleeding was present. Lymph nodes and the spleen of the recipient were markedly enlarged. Histology showed that the transplanted small bowel was almost normal except for a moderate, generalized infiltration of the lamina propria with lymphocytes and macrophages. The transplanted mesenteric lymph nodes were enlarged and severely depleted of lymphocytes. They consisted mainly of large macrophages and connective tissue cells. The recipient mesenteric lymph nodes and spleen were enlarged because of immunoblast proliferation. Splenomegaly was also caused by an increased hematopoesis. In the epithelia of the recipient tongue, esophagus, ear skin, and kidney pelvis, a dense subepithelial infiltration in the lamina propria was found. The infiltrating lymphocytes, macrophages, and dendritic-shaped cells showed a tendency to invade the epithelium across the basal lamina. In tongue and ear skin there was a focal denudation of the basal lamina with extravasation of granulocytes. These areas lay immediately adjacent to almost unaffected stratified epithelium.

Gross parenchymal damage was absent in the heart, liver, and pancreas, although a moderate infiltration of the periductular connective tissue was found in the latter two organs. This was also true for the recipient small bowel, where epithelial damage was generally absent in spite of a heavy infiltration in the lamina propria. Immunohistology showed a generalized induction of the expression of class II MHC antigens in the endothelia of large arteries and veins. Focally, class II MHC antigens appeared in normally negative epithelia of pancreatic and bile ducts. In the tongue, esophagus, and ear, dense accumulations of class II-positive cells were found immediately beneath, and also focally within, the epithelium. In most cases all of the layers of epithelial cells that were normally class II-negative turned positive. In the ear skin, class II MHC-positive cells were especially pronounced at the entrance of hair follicles.

In general, there was a marked increase in class II MHC-positive dendritic cells in all organs investigated.

RT1.A incompatibility

All recipients of an RT1.A-incompatible, semiallogenic, small-bowel transplant developed mild and temporary symptoms of GVHD between post-transplant days 25 and 40, including erythema of the ears and a mild, ulcerating dermatitis. In two animals the clinical picture progressively deteriorated, and they died 30 and 40 days after transplantation. Postmortem examination revealed a generally milder cellular infiltration in the organs than observed after a fully MHC-disparate small-bowel transplantation. The spleen was rather small and there was no increased hematopoesis in the red pulp. Also, the induction of class II antigens in epithelia, especially of the tongue, appeared only focally. The remaining animals survived the observation period of 100 days. Autopsy and paraffin histology showed no abnormalities. Immunohistology, however, showed very rare focal epithelial and subepithelial infiltrates of class II-positive cells in the tongue and ear skin. At these sites epithelial cells focally expressed class II MHC antigens.

RT1.B/D incompatibility

Class II MHC-incompatible small-bowel transplants induced an acute and lethal GVHD that did not differ from that observed after transplantation across a full MHC barrier. All recipients died within 20 days after transplantation (Table 3). Survival times of the recipients of class II and fully MHC-disparate small-bowel transplants did not differ significantly. On autopsy, histology, and immunohistology, the typical features of an acute and lethal GVH reaction were found, as already described.

RT1.C incompatibility

RT1.C-incompatible small-bowel transplants did not induce any GVH reactions in the recipient. No abnormal symptoms were present and the animals never differed from recipients of isogenic transplants. Autopsy and histology also showed no evidence of GVH reactions.

Discussion

This study clearly demonstrates that the development of GVHD after small-bowel transplantation is controlled genetically by the MHC. The histocompatibility barrier between the donor and the recipient determines the type and strength of GVH reactivity induced by small-bowel transplants. Whenever class II MHC incompatibility is involved, acute and lethal GVHD develops, while disparity of class I MHC antigens alone is usually associated with a milder, temporary, and usually nonlethal GVH reaction.

Class II MHC antigens, either alone or in combination with class I MHC antigens, induce an acute and lethal GVH reaction with characteristic clinical, autoptic, and histological features. The main physical signs are erythema and ulcerating dermatitis. Other symptoms, such as progressive weight loss, distended abdomen, and diarrhea also develop but are not pathognomonic for GVH reactions. This is shown by the fact that they also occur during rejection of small-bowel transplants [our own unpublished observations, 3, 8, 12].

The two basic histological features of acute GVHD are (1) infiltration of the recipient's subepithelial connective tissues by mononuclear cells, mainly in the gastrointestinal tract, the urogenital tract, and the skin, and (2) severe lymphadenopathy within the recipient's lymph nodes and spleen. Parenchymal organs like the heart, liver, kidney, and pancreas are not affected by significant interstitial infiltrates, and gross parenchymal damage is absent [3, 11–13, 16].

We would like to postulate that the main target of the transplanted lymphocytes is the recipient's lymphatic system [6]. The lymph nodes, the spleen, and the subepithelial lymphatic tissue of the gastrointestinal and urogenital tract, i.e., the mucosal-associated lymphoid tissue, as well as the Langerhans cells within the subepithelial connective tissue of the skin, are the victims of the transplanted lymphocytes. The GVH reaction induced by class II MHC-incompatible small-bowel transplantation is primarily not directed against non-lymphatic cells.

The severity of GVH reactions does not correlate with the expression of the recipient's class I and class II MHC antigens. Class II MHC antigens are, in fact, predominantly expressed on lymphoid and dendritic cells and only rarely on organ parenchyma, while class I MHC antigens are expressed on nearly all lymphoid and parenchymal cells [14]. Nevertheless, only class II MHC antigens are able to stimulate transplanted T lymphocytes sufficiently, while class I MHC antigens are not able to induce severe GVH reactions in the transplanted lymphocytes. During acute GVHD, expression of class II MHC antigens is induced on other nonlymphoid cells, which might then also be affected by GVHD.

An important question concerns the cause of death of the animals suffering from acute GVHD. All animals lost about one-third of their body weight within a few days. The weight loss was most likely due to the inability of the animals to eat and drink [15] because of the severe subepithelial infiltration in the entire gastrointestinal tract. The immediate cause of death of these completely emaciated animals was gastrointestinal bleeding and paralytic ileus. The bleeding was not only induced by the alteration of the gastrointestinal tract, but may have been a consequence of deranged hematostasis and thrombocytopenia [16].

Unlike class II MHC antigens, class I MHC antigens encoded by the RT1.A and RT1.C regions are not capable of inducing an acute and lethal GVHD. Recipients of RT1.C-disparate grafts showed no evidence of GVH reactivity. The animals displayed neither clinical symptoms nor histological abnormalities. Recipients of RT1.A-incompatible small-bowel transplants showed signs of ongoing GVH reactivity. These were essentially the same symptoms as those described in cases of class II MHC incompatibility. However, the GVH reaction is characterized by a delayed onset of symptoms with spontaneous resolution in most cases. Only two the six graft recipients died from GVHD on days 30 and 40. Even these animals survived longer than any recipient of a class II MHC-incompatible graft. Signs of chronic GVHD, such as alopecia and hyperkeratosis, [16] were not noted.

In conclusion, the strong effect of class II MHC antigens on the development of GVHD following semiallogenic small-bowel transplantation has been demonstrated. In allogenic transplantation in immunocompetent rats, class II MHC incompatibilities are very important as well. Following class II MHC-incompatible, allogenic, small-bowel transplantation, both GVH and host-versus-graft reactions occur simultaneously [9]. Therefore, GVH

reactions are not an artificial phenomenon after semiallogenic small-bowel transplantation. As for the implications of this study for clinical transplantation, class II MHC incompatibilities should be avoided. However, even class I MHC or non-MHC incompatibilities [4] may induce GVH reactions therefore, MHC matching alone is no guarantee against GVH reactions.

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