

MIGRATION AND ACTIVATION PATTERN OF SPECIALIZED DENDRITIC CELLS AFTER HETEROTOPIC SMALL BOWEL TRANSPLANTATION IN A GRAFT-VERSUS-HOST MODEL OF THE RAT.

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ABSTRACT

Besides specific cellular-mediated T cell responses, B cell related humoral responses have been demonstrated during the course of graft-versus-host disease after semiallogeneic transplantation of cellular antigen. Following semiallogeneic small bowel transplantation, there are, besides others, two specific forms of antigen-presenting cells, namely sinus lining cells (SLCs) and follicular dendritic cells (FDCs) which mediate primary and secondary humoral immune responses, respectively. This study was aimed to clarify the role of these dendritic cell entities after transplantation of small bowel grafts in a one-sided graft-versus-host (GvH) model for untreated and immunosuppressed (15-deoxyspergualin) recipient animals. As graft-versus-host disease progressed, SLCs and FDCs were eliminated in donor and recipient graft-versus-host associated target tissues (spleen and mesenteric lymph nodes) of untreated animals, whereas these dendritic cells prevailed in immunosuppressed recipients. 15-deoxyspergualin successfully prevented GvHD and significantly prolonged the mean survival time of untreated rats (16.0 ± 4.5 d) for at least 21 d. Based on the immunosuppressive efficacy of 15-deoxyspergualin on the survival and function of SLCs and FDCs, an unaltered development of germinal centers and B cell proliferation within mesenteric lymph nodes and spleen was maintained.

INTRODUCTION

One major mechanism in the initiation of allogeneic organ rejection is mediated by specialized antigen-presenting cells within the gut associated lymphatic tissues (GALT). Besides the commonly described professional antigen presenting cells (APCs) like Langerhans cells, veiled dendritic cells, and interdigitating dendritic cells, there are two unique forms of dendritic cells - namely sinus lining cells (SLCs) and follicular dendritic cells (FDCs) - which effectively contribute to the process of humoral

immune responses (1). SLCs, also designated as antigen transport cells (ATCs) (2,3), derive from bone marrow precursors (monocytes) (1,2) and are preferentially localized within the marginal and interfollicular sinus of lymph nodes and the marginal zone of the spleen. Here, their main function is to capture soluble antigen and particulate material which enters the afferent lymphatic vessels. After endocytosis of exogenous antigen, fragmented molecules are loaded as peptides into major histocompatibility complex (MHC) class-II specific molecules. Following antigen expression on their cell surface, SLCs migrate to the B cell dependent areas of lymph nodes, spleen, and Peyer's patches where they give rise to the differentiation of follicular dendritic cells and germinal center formation (4). In contrast to SLCs, FDCs preferentially present native antigen in the form of antigen-antibody-complexes which are retained in immunogenic form on their cell membranes to mediate antibody responses to exogenous antigens via B-cell proliferation. FDCs are non-phagocytic specialized dendritic cells which stimulate secondary humoral immune responses, including the clonal proliferation of memory B cells (5). It has been demonstrated that activated B-cells bind to FDCs, a process which is dependent on the interaction of cell surface adhesion molecules in addition to antigen contact (6). As FDCs are well endowed with complement receptors of all three types, they are able to target large amounts of immune complexes which continue to stimulate B-cells (7). The continuous antigen presentation in close proximity to B-cells is required for the formation of secondary germinal centers and the maintenance of immunological memory (8).

Following small bowel transplantation, a large number of donor-derived immunocompetent effector cells invade the lymphatic tissues of the host. Under circumstances where the host's immune defense mechanisms are compromised, host-specific effector cells of the graft are able to trigger a lethal graft-versus-host reaction (GvHR). In this study, a graft-versus-host model was established between inbred rat strains and heterotopic small bowel transplantation (HSBTx) was performed. The established graft-versus-host model was chosen to investigate the role of SLCs and FDCs in a one-sided rejection model, where immunocompetent cells of donor and recipient lymphatic tissues were supposed to demonstrate different migration and proliferation patterns due to a different recognition mechanism between parental

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and F1-hybrid derived immunocompetent cells. In this context, we hypothesized that 15-deoxyspergualin (15-DOS) which has been shown to suppress primary and secondary responses to *thymic dependent* and *independent antigens* (9) would be effective to sustain an unaltered functional and anatomical compartmentalization of the B-cell associated units of lymphatic organs during the course of a graft-mediated *anti-host* immune attack.

MATERIALS AND METHODS

In this study we established a semiallogeneic parent to F1 hybrid graft-versus-host model by use of male parental DA (RT1.^{av1}) inbred donor rats and female (LEW(RT1^b) x DA) F1-hybrid recipients. Heterotopic small bowel transplantation (HSBTx) was performed according to a modified technique of Monchik and Russell as previously published (10). Briefly, total small bowel was harvested with aortic cuff and portal vein, followed by perfusion with cold saline. For the revascularization, the graft aortic cuff was anastomosed to the infrarenal abdominal aorta. The graft portal vein was anastomosed to the infrarenal vena cava. The proximal gut lumen was closed. The distal end was inserted in an end-to-side fashion into the recipient's distal ileum. Animals (age between 16 and 20 weeks) were purchased at the "Zentralinstitut für Tierzucht Hanover, FRG" and weighed between 250-300g. Animal use and care respected the guidelines established by the German Council on Animal Care.

Animal Groups

Experiments included the following three groups (n=30):
Group 1: HSBTx of DA ⇒ F1, DOS 5mg/kg rat, from day 0 to day 14
Group 2: HSBTx DA ⇒ F1, no treatment
Group 3: HSBTx F1 ⇒ F1, no treatment

Group 1, 2, and 3 consisted of 4 subgroups of six animals which served to investigate the kinetic distribution pattern of various lymphocytes and dendritic cells (DCs) in the spleen and donor and recipient mesenteric lymph nodes at elective points of time: postoperative day 3, 7, 14, and 21 following small bowel transplantation.

Animal treatment

Lyophilized 15-deoxyspergualin was resuspended in 1 ml of saline (0.9%) and injected intramuscularly at a dose of 5mg/kg body weight from day 0 to day 14 following HSBTx.

Immunohistology

Small bowel grafts and mesenteric lymph nodes of donor and recipient together with spleen, donor and recipient mesenteric lymph nodes and small bowel

specimens were harvested on day 3, 7, 14 and 21 following SBTX. The tissues were sliced into small pieces, either snap-frozen in liquid nitrogen and stored at -80°C or subjected to 10% buffered formaldehyde, embedded in paraffin, cut at 4µm and stained with hematoxylin and eosin (H&E). Immunohistochemical analysis was performed using an indirect APAAP-staining technique (11). The panel of monoclonal antibodies (mAbs) used to characterize different lymphocyte populations included: Ki-M2R (macrophages), Ki-B1R (B-cells), Ki-T1R (pan-T cells), Ki-M4R (follicular dendritic cells) (12), and Ki-M9R (sinus lining cells) (1). After staining, the number of positive cells was determined and expressed as a percentage of the total cell count seen in 4 high-power fields per specimen. H&E-stained sections allowed the diagnosis of GvHD in recipient small bowel tissues as confirmed by the accepted histological parameters as described by Grant (9).

Flow cytometric analysis

At postoperative day 3, 7, 14, and 21 donor and recipient mesenteric lymph nodes and spleen were harvested from recipient animals to perform fluorescence activated cell sorting (FACS) analysis. For assessment of T and B lymphocytes and macrophages, monoclonal antibodies were used as follows: MRC OX19 (mouse-anti-rat pan T cells); MRC OX12 (mouse-anti-rat B cells) and MRC OX41 (mouse-anti-rat macrophages) purchased at Camon, Wiesbaden-Germany. Peroxidase-conjugated rabbit anti-mouse (Dakopatts, Glostrup, Denmark) and peroxidase conjugated affinity pure F(ab')₂ FITC-labeled goat anti-rabbit IgG (Dianova, Hamburg-Germany) monoclonal antibodies were used as secondary antibodies.

Statistics

Survival curves were generated according to the method described by Kaplan-Meier. The two-sided log-rank test was used to assess significant differences in animal survival.

RESULTS

Influence of 15-Deoxyspergualin Immunosuppression on Graft Survival

Treatment of F1-recipient rats with the immunosuppressant 15-deoxyspergualin (15-DOS) for 14 d, following transplantation of semiallogeneic parental (DA) small bowel grafts, significantly prolonged the mean survival from 16 ± 4.5 d to 39 ± 11 d. Figure 1 illustrates the survival curves of transplanted animals which were generated according to the Kaplan-Meier procedure. 15-DOS significantly prolonged the life expectancy of F1-recipients in comparison to untreated animals (p<0.0001, log-rank test). Table 1 summarizes individual survival of all transplanted animals from group one to three.

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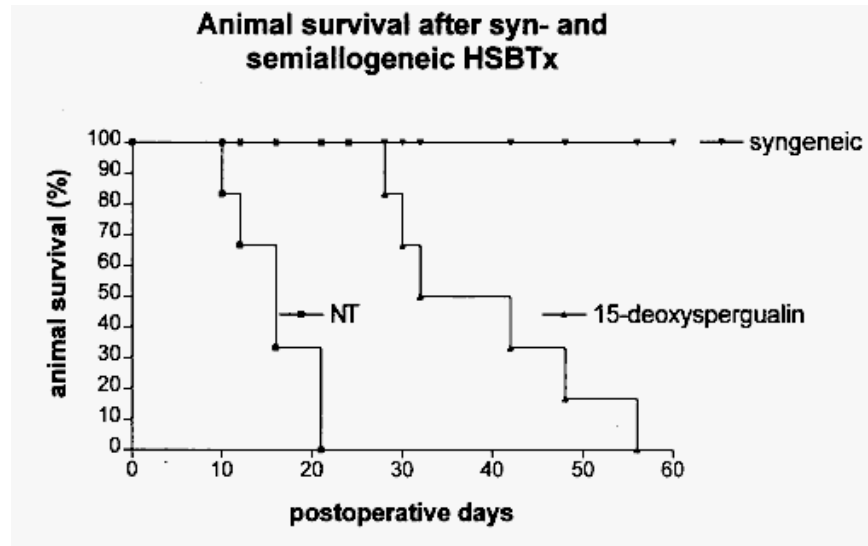


Figure 1: Survival of syngeneic [F1 → F1], non-treated (=NT) [DA → F1], and 15 deoxyspergualin (=DOS) immunosuppressed recipient rats [DA → F1] is illustrated. Kaplan-Meier analysis was performed to generate the specific survival curves. Statistical analysis with the Savage (Mantel Cox) log-rank test showed a significant difference in survival between untreated and immunosuppressed animals, $p < 0.001$ and between semiallogeneic and syngeneic F1-hosts, $p < 0.0001$.

Table 1: Animal Survival after Heterotopic Semiallogeneic Small Bowel Transplantation

Experimental Group	Treatment	Survival	Mean (d)
I. DA ⇒ F1	none	10, 12, 16x2, 21x2	16 ± 4.5
II. DA ⇒ F1	15-DOS (5mg / kg, i.m., d 0 -14)	28, 30, 32, 42, 48, 56	39 ± 11*
III. F1 ⇒ F1	none	100x6	> 100

*Survival of 15-deoxyspergualin (=DOS) immunosuppressed animals differed significantly from non-treated animals of group 1 ($p < 0.0001$; two-sided log-rank test).

Gross examination of animals suffering from GvHD

Non-immunosuppressed F1-recipient animals subjected to parental donor grafts (group 1) suffered from mild to severe GvHD which started around postoperative day (POD) 8. Typical manifestations of GvHD included weight loss (20% or greater of preoperative body weight), skin rash, cachexia, diarrhea, altered gait, and hunched posture. In contrast to non-immunosuppressed animals, none of the DOS-treated animals or animals which had received syngeneic grafts suffered from these symptoms during the first 14 postoperative days. When 15-DOS administration was stopped after 14 days, all recipient animals started to suffer from signs of graft-versus-host reactions. However, in contrast to untreated F1-hosts, the clinical course of GvH development was substantially prolonged and lethal graft-versus-host disease did not occur before POD 28 and 56, as outlined on table 1.

Histological evaluation

In this study, the distribution, activation and

compartmentalization of SLCs and FDCs and their impact on the development of primary and secondary lymph follicles and germinal center formation within donor and recipient mesenteric lymph nodes and spleen were of special interest. The correlation between the extent of GvHD and the frequency and distribution pattern of SLCs and FDCs will now be described in detail.

Sinus Lining Cells (SLCs) are Activated within the Afferent Lymphatic Vessels of Donor and Recipient Mesenteric Lymph Nodes and Host Spleen Following HSBTx

Transplantation of DA parental small bowel grafts into F1 hybrids (LEW x DA) induced a considerable alteration of the distribution pattern of immunocompetent cells within the afferent lymphatic vessels (marginal and interfollicular sinus of lymph nodes and marginal zone of the spleen) and adjacent B cell compartments. Until day 7 following HSBTx, there was a marked increase of

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KiM9R⁺ SLCs in both, untreated and immunosuppressed recipients in comparison to syngeneic transplanted animals as summarized on figures 2A, B, and C. Following transplantation of parental small bowel into F1-hybrid recipient rats, the frequency of SLCs was highly increased within the marginal sinus of donor and recipient mesenteric lymph nodes and within the marginal zone of the spleen. Figures 5a and 5b depict the activation pattern of SLCs from a non-immunosuppressed animal at POD 7. As illustrated, SLCs surround the primary follicles in both, recipient (Figure 5a) and donor mesenteric lymph nodes (Figure 5b), respectively. In mesenteric lymph nodes of both, donor and recipient origin, SLCs were mainly localized within the afferent lymphatic vessels for the first three days following transplantation. They, then, started to migrate to the interfollicular space and surrounded the cortical follicles. Here, they emerged through the cell web formed by lining cells of the lymphatic sinus and could be visualized immunohistochemically in the center of the primary follicles. Figure 5c shows a SLC stained with KiM9R and coupled to an immunofluorescence dye (cytochrome-3) in the middle of a germinal center of a donor mesenteric lymph node (POD 7). The kinetic of this migration pattern was quite different in the syngeneic strain combination, where the frequency of SLCs within the afferent lymphatic vessels of the spleen and lymph nodes did not exceed the control levels of non-transplanted and age-matched F1-hybrid rats. After syngeneic SBTx, the percentage of SLCs found within the center of primary follicles was significantly less than observed in the semiallogeneic strain combination. In coincidence with the observed invasion of primary follicles by SLCs, activation of the germinal centers ensued and the number of follicular dendritic (KiM4R⁺) cells rose significantly.

In small bowel grafts, SLCs lined up around the peyer's patches and were found sporadically within the lamina propria in animals of groups 1 and 2, whereas syngeneic control animals did not demonstrate such an influx of SLCs during the ultimate postoperative period (POD 3 to 7) (data not shown). In line with the progression of graft-versus-host disease which started to become apparent clinically between days 8 to 12 following SBTx, untreated and immunosuppressed recipient animals revealed quite a different distribution pattern of SLCs within the investigated B cell compartments of mesenteric lymph nodes and spleen. As evident from figure 5d, the frequency of SLCs was significantly diminished in the spleen of non-immunosuppressed animals at POD 14 following SBTx. At this point of time, an accurate assignment of SLCs to the distinguished B cell compartments of the spleen was impossible, since all germinal centers and their surrounding perfollicular areas had lost their typical picture, caused by a substantial

depletion of functional lymphocytes during the course of progressing GvHD. In contrast, animals which had been treated with 15-deoxyspergualin for the first 14 postoperative days did not reveal these detrimental destruction of the various functional lymphatic units within the spleen as demonstrated on figure 5e. Figure 5e shows a normal distribution and density of SLCs which are localized mainly around activated secondary germinal follicles of the spleen. For comparison, figure 5f represents a spleen specimen harvested at POD 14 after syngeneic small bowel transplantation. Figures 2A-C summarize the migration kinetics of SLCs within the described functional B cell units of spleen and mesenteric lymph nodes, respectively. It is evident that non-immunosuppressed animals which suffered from lethal GvHD showed a significant decline of KiM9R⁺ sinus lining cells as graft-versus-host disease progressed. Comparison of donor and recipient mesenteric lymph nodes showed an apparent difference of the activation pattern of SLCs within the marginal sinus of their lymphatic vessels. Whereas the peak of SLC-activation in host mesenteric lymph nodes was reached within the first three postoperative days, the maximum increase of this cell type was delayed until POD 7 in donor mesenteric lymphatic vessels.

Proliferation of Follicular Dendritic Cells and Subsequent Development of Secondary Follicles was Suppressed as Graft-versus-Host Disease Progressed

Phenotypic characterization of follicular dendritic cells was performed by the use of Ki-M4R, a highly specific monoclonal mouse-anti-rat antibody. Whereas at POD 3, after semiallogeneic HSBTx, the frequency of FDCs was not increased in splenic follicles of recipient animals, (Figure 6a), the number of positively stained Ki-M4R⁺ cells was significantly augmented in recipient mesenteric lymph nodes in comparison to syngeneically transplanted F1-hosts, figure 3B. In non-immunosuppressed animals, this proliferative response of FDCs could be observed in the follicles of donor and recipient mesenteric lymph nodes until POD 7, as illustrated on figure 3B and 3C, respectively. After that point of time, growth and numeric increase of FDCs within germinal centers were inhibited. Interestingly, consistent with the activation pattern and kinetics of SLCs, peak numbers of FDCs were observed at POD 3 in the host mesenteric lymph follicles, whereas a comparable increase of FDCs was first evident at POD 7 in the mesenteric lymph nodes of donor origin. Comparison of the kinetics between SLCs (Figures 2a-c) und FDCs (Figures 3a-c) reveals a strong correlation between both cell types as to their common activation patterns within the various lymphatic tissues. As it

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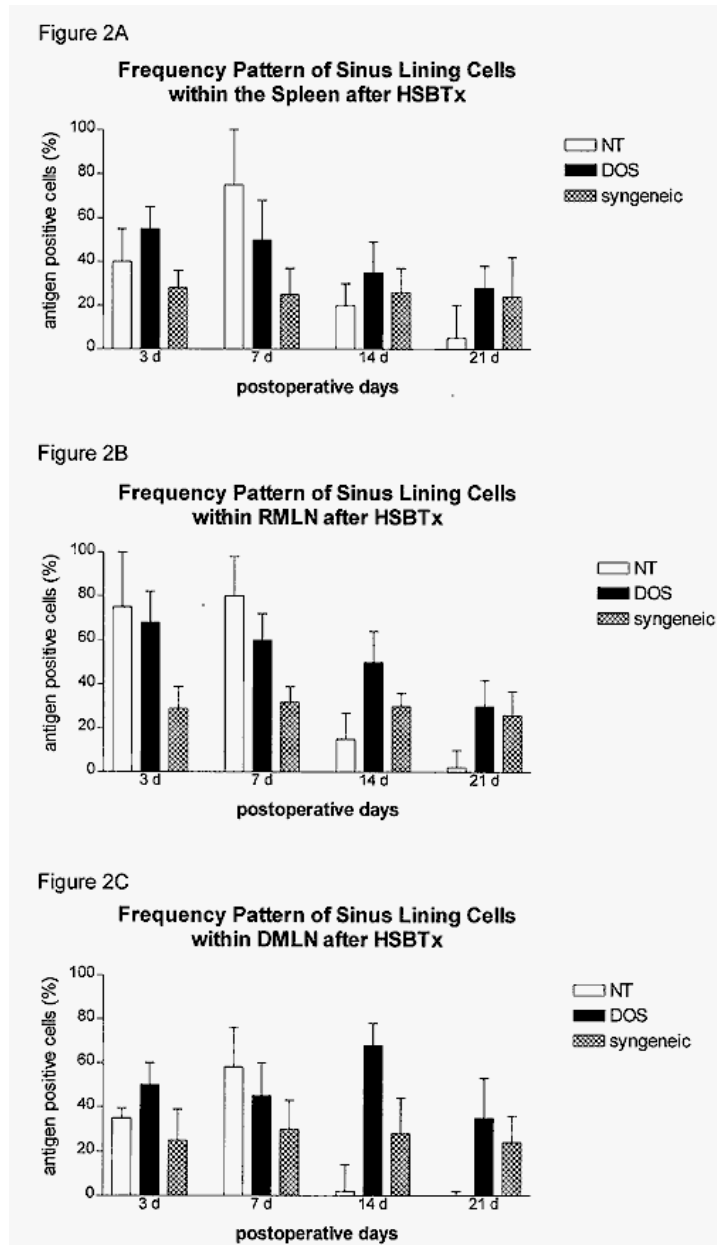


Figure 2 A-C

A. The frequency pattern of KiM9R⁺-stained sinus lining cells [SLCs] was assessed in spleen specimens at various points of time after heterotopic small bowel transplantation. Open bars (non-treated = NT), solid bars (immunosuppressed, 15-deoxyspergualin = DOS), and hatched bars (syngeneic) represent mean ± SD of 4-6 adjacent high power fields examined for each marginal zone of recipient spleens from 2-3 rats. Values are presented as the number of antigen-positive cells / the number of all visible cells x 100.

B. Shows the frequency pattern of SLCs within the marginal sinus of recipient mesenteric lymph nodes at various time points after heterotopic small bowel transplantation. Relative numbers were evaluated as described above.

C. The relative numbers of SLCs from donor mesenteric lymph nodes at various time points after heterotopic small bowel transplantation is indicated, as described above.

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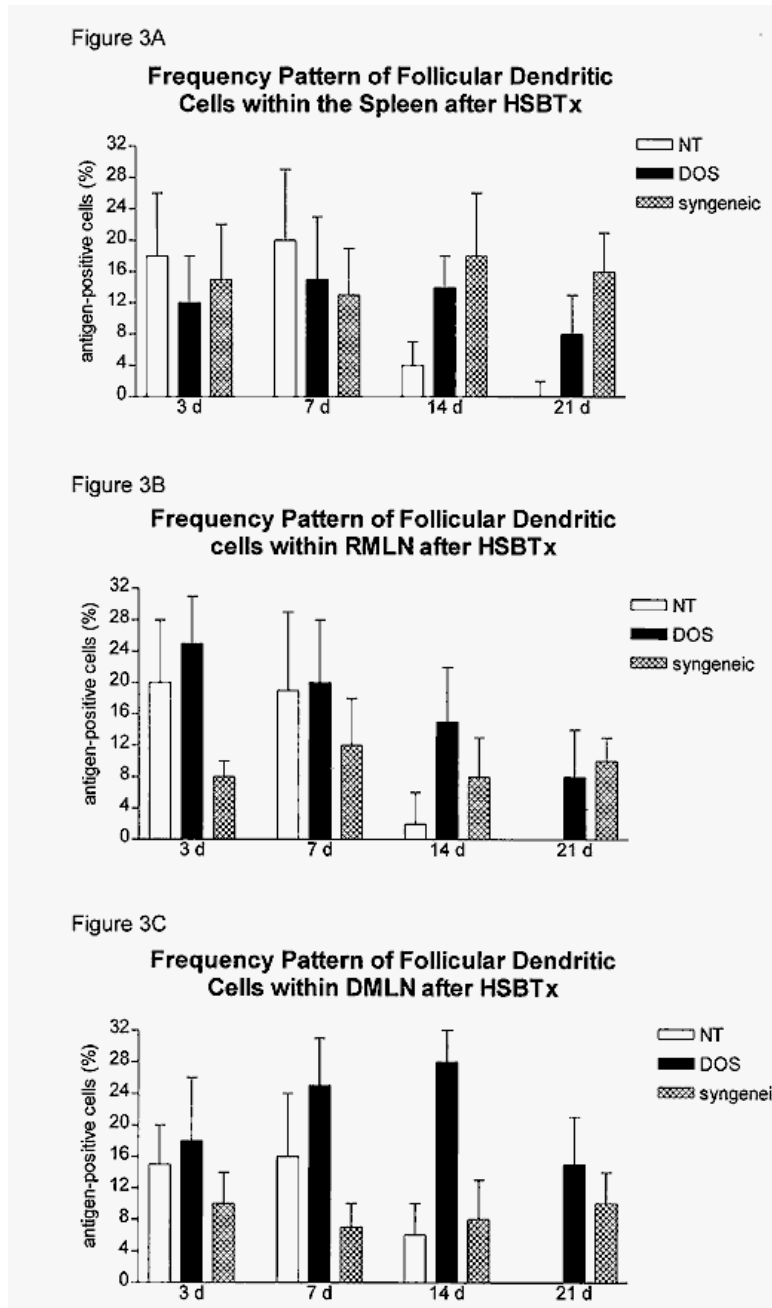


Figure 3 A-C

A. The frequency pattern of KiM4R⁺-stained follicular dendritic cells [FDCs] was assessed in spleen specimens at various points of time after heterotopic small bowel transplantation. Open bars (non-treated = NT), solid bars (immunosuppressed, 15-deoxyspergualin = DOS), and hatched bars (syngeneic) represent mean \pm SD of 4-6 adjacent high power fields examined for each marginal zone of recipient spleens from 2-3 rats. Values are presented as the number of antigen-positive cells / the number of all visible cells x 100.

B. Shows the frequency pattern of FDCs within primary and/or secondary follicles of recipient mesenteric lymph nodes at various time points after heterotopic small bowel transplantation. Relative numbers were evaluated as described above.

C. The relative numbers of FDCs from donor mesenteric lymph node follicles at various time points after heterotopic small bowel transplantation is indicated, as described above.

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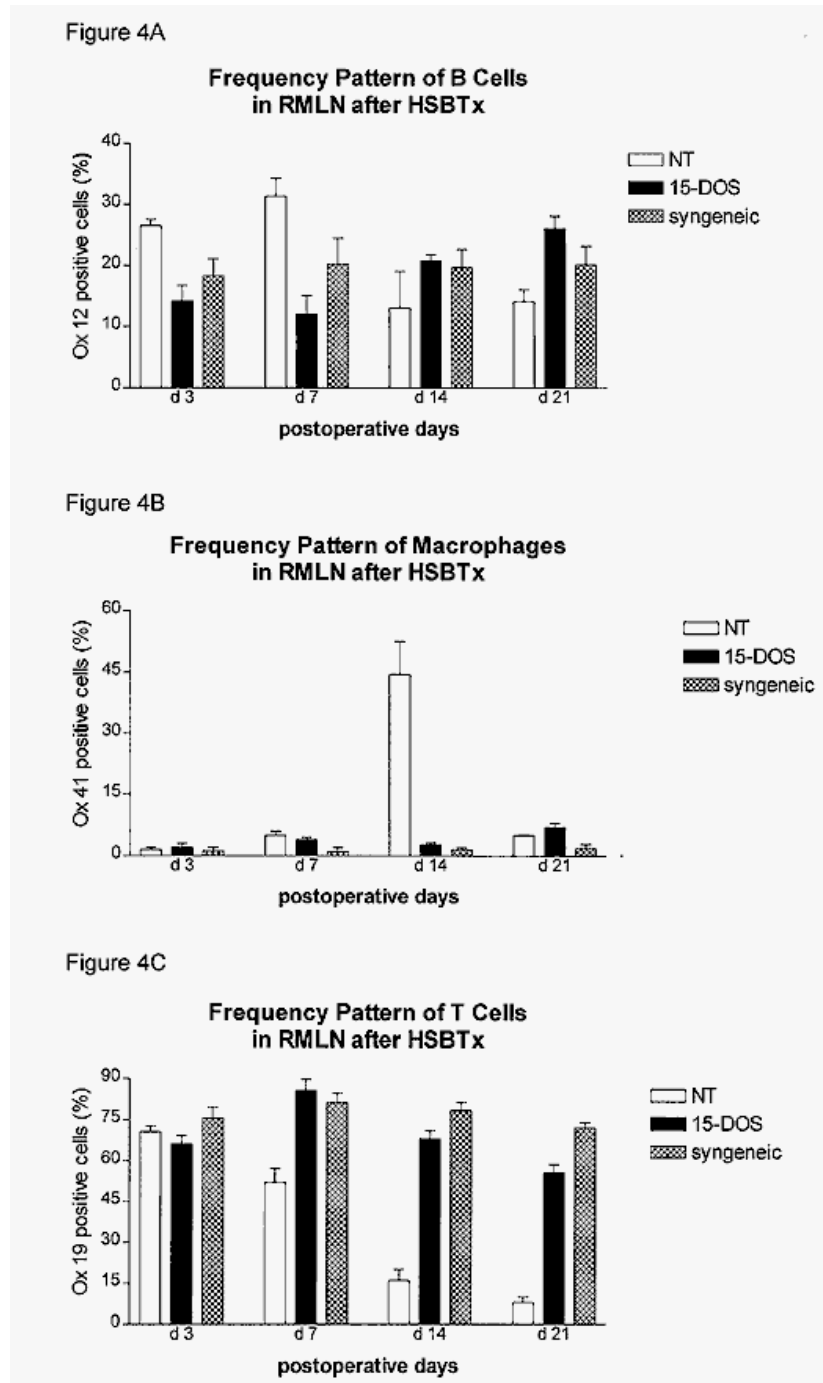


Figure 4 A-C

Figure A: Flow cytometric analysis was performed from spleens of nontreated (= NT) immunosuppressed (15-deoxyspergualin = DOS) and syngeneically transplanted F1 hosts at various time points after HSBTx. The relative number of B cells stained with mAb Ox-12 was assessed in four individual experiments and is given as mean ± SD. Simultaneously, the relative number of Ox-41⁺-cells (macrophages) [Figure B] and of Ox-19⁺-cells (pan-T cells) [Figure C] is displayed.

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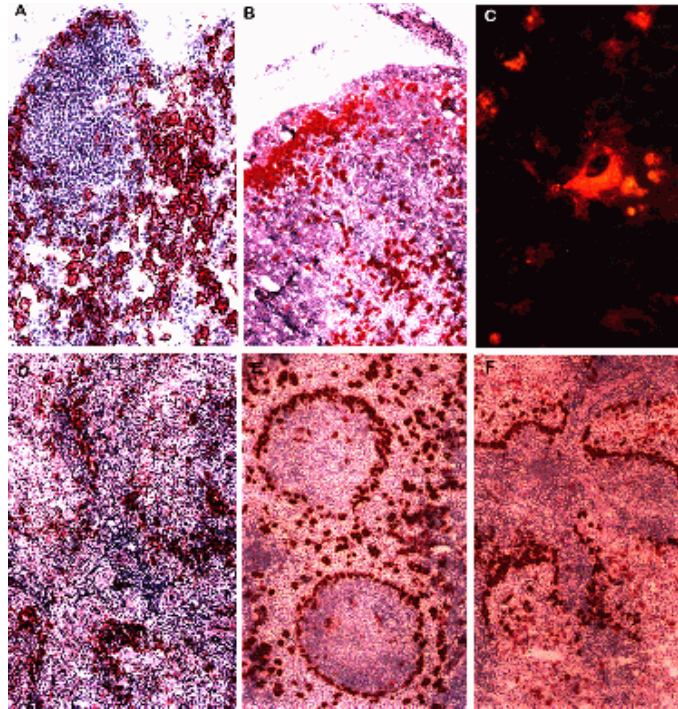


Figure 5 a-f

The distribution of KiM9R⁺-cells within the marginal and interfollicular sinus of recipient (Figure 5a x 70) and donor mesenteric lymph nodes (Figure 5b x 35), seven days after HSBTx is illustrated. Figure 5c (x 300) shows an immunofluorescent (cytochrome-3) staining of a KiM9R⁺-sinus lining cell which was visible in the middle of a germinal center, 7d after heterotopic small bowel transplantation. Figure 5d (x 35) and Figure 5e (x 35) represent the distribution pattern of SLCs taken from splenic specimens of untreated and immunosuppressed animals at postoperative d 14 after heterotopic small bowel transplantation. As illustrated, untreated animals suffered from a severe damage of the normal compartmentalization within the spleen, whereas SLCs of 15-deoxyspergualin immunosuppressed animals showed the normal distribution pattern of SLCs within the marginal zone and adjacent parafollicular units, at postoperative day 14. Figure 5f (x 35) displays the frequency and distribution pattern of SLCs from a splenic specimen 14 d after syngeneic small bowel transplantation.

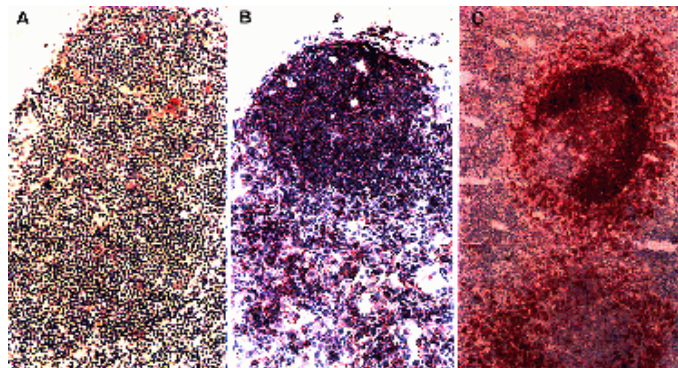


Figure 6 a-c

The distribution and frequency pattern of KiM4R⁺-follicular dendritic cells within recipient mesenteric lymph nodes is exemplified for a non-immunosuppressed (Figure 6a x 70) and a 15-deoxyspergualin treated animal (Figure 6b x 70), 14 d after heterotopic small bowel transplantation. Whereas immunosuppressed animals displayed the typical web-like structure of FDCs which comprised the whole follicle, no FDCs were found in lymph follicles of animals suffering from the progression of graft-versus-host disease. A normal FDC distribution pattern was associated with a normal B cell proliferatin pattern as illustrated on Figure 6c (x 35), a specimen taken from the spleen of a 15-deoxyspergualin-treated animal and stained with KiB1R-mAb, 14 d after small bowel transplantation.

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was true for SLCs, further progression of GvHD induced a progressive decline of the follicle integrity and concomitant depletion of FDCs in the spleen and mesenteric lymph nodes (Figure 3). In contrast, short-term administration of 15-deoxyspergualin inhibited acute GvHD in all F1 recipient rats for at least the first 4 postoperative weeks. The immunosuppressive action of 15-DOS was mirrored in an unaltered development of primary and secondary germinal follicles with concomitant germinal center formation following transplantation of parental (DA) small bowel. Subsequently, rising numbers of FDCs were observed in the spleen and lymph nodes of donor and recipient origin as depicted on figures 6. Figures 6a and 6b give evidence for the different staining pattern of FDCs which was found in host mesenteric lymph nodes of untreated and immunosuppressed animals, respectively, at POD 14. Whereas no FDCs were detectable in the cortex follicles of untreated animals at that time point, 15-DOS treated animals revealed the normal web-like pattern of FDCs which covered almost all of the transverse section of the depicted follicle. Besides, some smaller clusters of Ki-M4R⁺ cells were visible in the perifollicular area of these lymph nodes. As exemplified in Figure 6c which shows a B cell follicle of the spleen at POD 14 following semiallogeneic small bowel transplantation, the B-cell compartments of 15-deoxyspergualin treated animals showed a normal activation pattern within the spleen, the donor, and the recipient mesenteric lymph nodes which could also be confirmed by flow cytometric analysis of these tissues (next section). Conversely, non-immunosuppressed animals did not show functional B-cell compartments in these lymphatic tissues at that time point, neither morphologically nor immunohistochemically.

Flow Cytometric Analysis of Immunosuppressed and Non-treated F1-Recipient Animals Differed Significantly

To further assess the impact of GvHD-associated immunosuppression on the lymphocyte pattern within mesenteric lymph nodes and spleen, flow cytometric analysis was performed to distinguish the frequency of T and B cells and macrophages during the course of progression of graft-versus-host disease. As exemplified for recipient mesenteric lymph nodes in figures 4a-c, GvH-mediated tissue injuries of these target organs induced a significant elimination of B and T cells as GvHD progressed. Conversely, macrophages were attracted to these sites of destroyed B and T cell compartments of recipient mesenteric lymph nodes (Figures 4a and 4c). At postoperative day 14, there was a significant increase in the number of invading macrophages (Figure 4b) (KiM2R⁺-stained cells) preferentially residing in the cortex and paracortex of the destroyed lymph nodes. Similar findings were noted in the donor mesenteric lymph nodes and spleen, however with a less rapid decline of B and T cells (data not shown).

DISCUSSION

Various studies have emphasized the rapid mutual exchange of donor and recipient lymphocytes between graft and host lymphatic tissues following small bowel transplantation (14). In the experimental setting of a GvH-model, *donor* lymphocytes are activated in the environment of the recipient's lymphatic organs (central sensitization), whereas *host* lymphocytes stimulate an allospecific *anti-host* immune response after migration to the lymphatic tissues of the graft, namely, lamina propria and Peyer's patches of the small bowel and adjacent mesenteric lymph nodes (peripheral sensitization) (15).

The observed pattern of sequential proliferation of *host*- and *donor*-derived sinus lining cells, and then by follicular dendritic cells sheds new light on the firm correlation between bone marrow derived sinus lining cells and follicular dendritic cells. The origin of FDCs is still strongly debated, as earlier studies identified this cell entity to belong to the reticular cell type derived locally from primitive reticular cells or from mesenchymal cells (pericytes around capillaries) (16,17). Experiments by Humphrey *et al.* (16) who investigated bone marrow chimeras demonstrated that FDCs were of host origin, thus supporting the notation of a reticular dendritic cell type. In contrast, Pawaresch *et al.* (18), Wacker *et al.* (1), and Szakal *et al.* (2,3) have demonstrated cells with a follicular dendritic phenotype in human, rat, and mouse blood, respectively. From their experiments, it was concluded that follicular dendritic cells may originate from blood cells derived from bone marrow. Kapasi *et al.* clearly provided support for the bone marrow as being a source of FDC precursors by the use of SCID mouse-rat and SCID-mouse F1 chimeras (19).

In line with findings by Szakal *et al.* (2,3) for the mouse species, Wacker has demonstrated for the rat a similar dendritic cell type of monocyte origin by the use of autoradiographic labeling studies (1). This dendritic cell was designated, sinus lining cell, as it was mainly localized in the subcapsular sinus floor where one of its functions was envisioned to mediate antigen trapping and presentation. Migration of SLCs to the follicle cortex was postulated, since SLCs could be traced in the follicle center by the use of electron microscopy.

The findings in this paper give direct evidence that a specific immunogenic stimulus leads to activation of SLCs in host mesenteric lymph nodes within the first 72 hours after reperfusion of the grafted organ. The fact that host-derived SLCs were activated by donor-derived immunocompetent cells entering the afferent vessels of the recipient's mesenteric lymph nodes can be assumed for the following reasons: host-derived (F1) immuno-competent cells which circulate through the graft will be recognized as non-self and will give rise to a specific *anti-host*

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response by graft-derived effector cells. Conversely, these circulating host lymphocytes and monocytes will not recognize graft cells as *non-self* as they carry one half of the parental genome themselves. Moreover, activated passenger leukocytes released by the graft enter the host lymphatic tissues and elicit a direct *anti-host* immune response. The apparently immunogenic character of the observed proliferation and migration pattern of the host-derived SLCs is strengthened by the fact that syngeneic grafts did not trigger an activation and did not reveal an increase of the number of SLCs in the host lymphatic organs. For these reasons, it is justified to conclude that activation of the SLCs within host lymphatic organs was due to the interaction of invading graft-derived cells or graft-derived soluble antigens. In this context, it is noteworthy that the graft-associated immune attack against the host tissues simultaneously induced an activation and proliferation of the immunocompetent dendritic cells of the host's immune arm.

Whereas 15-deoxyspergualin warranted a mutual and sustained antigen exchange between activated DCs in the graft and within the host, progression of the graft-versus-host reaction led to a substantial damage of certain GvH-related target organs, such as spleen, lymph nodes, liver, skin, small bowel, and thymus. Thus, a second step of host tissue destruction ensued the initial activating stimulus. However, 15-deoxyspergualin immunosuppression effectively inhibited the GvH-mediated injury to B and T cell compartments of the secondary lymphatic organs and conferred a permanent activation of B cell follicles with consecutive formation of germinal centers, which was not observed in the non-immunosuppressed animals.

B cell proliferation, differentiation, and affinity maturation has been linked to an intact interaction between virginal B cells and the specific effector cells of the activated (secondary) follicles, such as centrocytes, centroblasts and FDCs. As soon as immunogenic antigens invade the body, specific antibodies against these antigens are formed which give rise to antigen-antibody complexes. Most of these immune complexes are ingested by the mononuclear phagocytes. A small part, however, will be trapped on the surfaces of cell processes protruding from the follicular dendritic cells (20,21). It has been emphasized that these FDC-immobilized antigen-antibody complexes play a crucial role in the generation of memory B cells (21) and in the phenomenon of "affinity maturation" (22). It is worthwhile to mention, in this context, that antigen complexed with antibody can induce germinal centre proliferation of B cell memory without evoking a detectable antibody response (23). Petrasch *et al.* demonstrated that isolated FDCs appear to stimulate the proliferation of B-cells *in vitro* while suppressing immunoglobulin G (IgG) production at the same time (24). Taken together, there is now strong evidence, that the mechanism of antibody mediated generation of B-cell

memory and "affinity maturation" on one side and the mechanism of enhancing antibody responses on the other side might well be orchestrated by two quite different pathways.

Immunosuppressed animals which keep the balance between donor and host specific antigen presentation mediated by specialized dendritic cells are prone to long-term survival and graft acceptance (25). SLC's and FDCs appear to sustain normal B-cell development within the lymphatic follicles, which in turn is necessary for an adequate humoral response and an efficient cell-mediated immunogenic response via activation of CD4-helper T-cells within the T-cell dependent areas. Whether this balanced antigen presentation by specialized dendritic cells is the basis for the stable microchimerism following vascularized solid organ transplantation, as speculated by Starzl *et al.* (25), will be subject of further long-term investigations.

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