Graft-versus-Host Reaction and GvH disease

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Chronic GvHR was induced by inoculating parental lymphoid cells into F1 hybrid mouse. Combination of ATL and ATH, which were congenic recombinant strains differing only in H-2I and S region from each other, was chosen to induce class II-GvHR. Selective activation against partner's alloantigen of graft CD4+ T cells was the primary event of the GvHR and then led to concomitant activation of both graft and host cells. Immune dysregulation among these cells made the GvHR-mouse express various chronic diseases including immune complex glomerulonephritis, autoimmune-like lesions of the liver or the salivary gland, tumor-like proliferations of T cells and abnormal extramedullary hematopoiesis. Chronic GvHR was also induced by a preferential but not a selective activation of graft CD4+ T cells. A combination of DBA/2 and C57BL/6, which differ in whole MHC antigens, was an example. When D2 cells, but not B6 cells, were inoculated into the BDF1 mouse, predominant activation of CD4+ cells over CD8+ cells were observed. Contributing factors to this phenomenon were low responsiveness of graft CD8+ T cells to allogeneic class I MHC antigens and anti-parent activity of host CD8+ cells. Thus both graft and host cells participate either actively or passively in the reaction induced in the parent → F1, experimental system of GvHR/D. ——— GvHR; GvHD; parent → F1; immune dysregulation

Lymphocyte recognition of the histocompatibility antigen occurs upon organ transplantation if the immune system has not been compromised. The direction of the recognition could be either host versus graft or graft versus host, and the reactions provoke profound clinical problems, i.e., rejection of transplant or graft versus host disease (GvHD), respectively. GvHD is well recognized as a major impediment to the therapeutic success of allogeneic bone marrow transplantation. It is clinically divided into acute and chronic diseases depending upon the time of occurrence and the duration of symptoms (Sullivan 1986). The major organs involved are the skin, liver and gastrointestinal system. The immune system of the host is also one of the target organs involved in GvH reaction (GvHR), which elicits either immunodeficiency or an autoimmune-like syndrome by the immunosuppressive or -stimulative effect of GvHR, respectively. Thus GvHR/D is not a simple immunological event but a series of complex biological events involving...
both graft and host cells and tissues.

To analyze these complex events, a variety of experimental systems for GvHR had been developed. The host versus graft reaction is avoided in these systems by the particular state of the immune system of the host, such as immaturity in the neonatal host, radio-destruction in an irradiated host or tolerance to parental alloantigens in an F1 host. Classical Simonsen's assay had been utilized to analyze the principal cells that were responsive to induce GvHR (Simonsen 1985). GvHR observed in a sublethally irradiated host was particularly suitable for observing GvHR occurring against minor histocompatibility antigen (Korngold and Sprent 1987), and therefore a good experimental model for GvHR/D occurring after human major histocompatibility (MHC) antigen-compatible bone marrow transplantation (Nonomura et al. 1987). GvHR in parent → F1 system, on the other hand, is unique in that the F1 host is an intact and not an immunologically treated mouse. This means that not only primary responding graft T cells but also secondarily activated host cells are involved in GvHR, which makes the feature of GvHD more complex (Harada 1990).

MHC antigen-, and not minor histocompatibility antigen-, incompatible combination of mice is employed in parent → F1 system (Gleichmann et al. 1984). Thus class I-, II- and (I+II)-GvHR are those observed across class I-, II-, or whole MHC antigen differences, respectively. GvHD elicited by these GvHRs has characteristic features, and the different nature of GvHD depends upon both the class of GvHR and the combination of mouse strains. When CD8+ T cells are predominantly activated in class I- or (I+II)-GvHR, symptoms of immunodeficiency, which are some of the important features of acute GvHD, become apparent (Harada et al. 1990). The reactivity of T cells to class I MHC antigen, however, is genetically different among mouse strains (Sprent and Schaefer 1989), thus the grade of resultant GvHR/D becomes varied among systems of GvHR employed. On the other hand, the dominance of alloantigen activated CD4+ T cells in class II- or (I+II)-GvHR leads to chronic GvHD, the clinical features of which show a great variety of symptoms, including autoimmune-like syndromes (Gleichmann et al. 1984; Harada et al. 1987).

In this report the features of chronic GvHR/D that we are investigating are summarized. One of two experimental systems we employed is a class II-GvHR, in which a combination of H-2I and S region-incompatible congenic recombinant strains, ATH and ATL, was used (Harada et al. 1987). The other system we used was parent → (C57BL/6 × DBA/2) F1 (BDF1). It is possible in this experimental GvHR system to observe either acute or chronic GvHR/D by selecting a graft strain, i.e., C57BL/6 (B6-GvHR) for the former and DBA/2 (D2-GvHR) for the latter (VIA and Shearer 1988). Although GvHR/D is elicited primarily by alloantigen reactive graft T cells, we focused on the active participation of host T cells in D2-GvHR to express the chronic GvHR.
**Induction of class II-GvHR**

Using parent \( \rightarrow \) F\(_1\), GvHR different types of GvHR could be induced by combining a pair of mouse strains among A/J, ATL and ATH. Class I-GvHR expressed in (ATL \times A/J) F\(_1\) (H-2K\(^{k/k}\) S\(^{k/d}\)) host was observed as a minimal splenomegaly reaction up to 6 weeks after iv. injection of parental spleen cells. On the other hand GvHRs of class I+II type elicited in (A/J \times ATH) F\(_1\) (H-2K\(^{k/s}\) I\(^{k/s}\) S\(^{d/s}\)) and of class II type in (ATL \times ATH) F\(_1\) (H-2I\(^{k/s}\) S\(^{k/s}\)) host showed the same level of splenomegaly up to 2 weeks of GvHR but the former resulted in lethal GvHD by the 6th week in the conventional feeding conditions whereas the latter showed further splenomegaly reaction (Table 1).

Splenomegaly induced by class II-GvHR in our experimental system showed essentially similar results with graft cells from either parental strain, or when the F\(_1\) host was either LHF\(_1\) or HLF\(_1\). Kinetic study revealed that the spleen weight continued to increase up to the 7th week after induction of GvHR. The reaction was dose-dependent and this was apparent even at the 13th week of GvHR.

**Cellular events in class II-GvHR**

That CD4\(^+\) T cells were principal responding cells in the class II-GvHR was confirmed by the deletion of CD4\(^+\) cells from the inoculating cell population by treatment with anti-L3T4 monoclonal antibody plus rabbit complement. The number of CD4\(^+\) cells in the GvH spleen was increased by the reaction, but the proportion of them in the organ did not change drastically during the early period of GvHR. Histopathological examination of GvH spleen showed the expansion of white pulp for the 1st week. It was revealed by immunohistochemical and FACS analysis that not only CD4\(^+\) but also CD8\(^+\) T cells or B cells proliferated in association with alloantigen reactive T cells (Fig. 1 and Table 2). That the number of host B cells was increased concomitantly was further confirmed either by double staining on FACS or by inoculating purified parental T cells instead of B cell-containing whole spleen cells. This clearly shows that not only graft cells

<table>
<thead>
<tr>
<th>Host</th>
<th>Spleen weight (mg)</th>
<th>Survival at 6 wk. (%)</th>
<th>Genetic difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>((\text{ATH} \times \text{A/J}) \text{ F}_1)</td>
<td>77 176 237 (118)</td>
<td>11</td>
<td>H-2K, I, S and background</td>
</tr>
<tr>
<td>((\text{ATH} \times \text{ATL}) \text{ F}_1)</td>
<td>72 166 255 312</td>
<td>100</td>
<td>H-2I, S</td>
</tr>
<tr>
<td>((\text{ATL} \times \text{A/J}) \text{ F}_1)</td>
<td>72 82 87 81</td>
<td>100</td>
<td>H-2K, S and background</td>
</tr>
</tbody>
</table>

5 \(\times 10^7\) parental spleen cells were injected iv into F\(_1\) mouse. Each of parental stains was examined in sex-matched combinations.
but also host cells participate in the GvHR.

Features of GvHD of our class II-GvHR

Immune complex glomerulonephritis. Not only the number of B cells but also that of immunoglobulin-producing cells in the spleen increased following the induction of GvHR. This resulted in long-lasting hypergammaglobulinemia in the host mice. The levels of serum IgM, IgG₁ and IgG₂a were 2.7 to 1.2 times higher than those of the control mice even at the 8th week of GvHR (Harada et al. 1988). The presence in the serum of these mice of various autoantibodies including anti-nuclear and anti-double strand DNA antibodies was also confirmed.
These autoantibodies were produced by the B cells which were polyclonally activated by various cytokines released by the activated T cells (Dobashi et al. 1987). Prominent proteinuria was observed clinically at and after the 3rd week of GvHR. This led to nephrotic syndrome in the later stage of chronic GvHD and caused death of mice from renal failure (Fig. 2). This was due to the formation of immune complex in the circulation and the resultant lupus-like glomerulonephritis. The hypergammaglobulinemia and IC formation, however, seemed to be not enough to form renal lesions. This was because we observed that the hypergammaglobulinemia, which was induced in (NZB x NZW) F1 mice by injecting parental CD4+ T cells, lasted more than half a year but did not accelerate the severity of spontaneous formation of lupus-like glomerular lesions in these F1 mice.

Other investigators also showed that the renal disease was most easily found in BDF1 mice when compared with other F1 mice produced by mating DBA/2 and other parental strains, although the same level of hypergammaglobulinemia was induced by GvHR in these F1s (Portabova et al. 1988). This indicates that renal factor(s) might also participate in the formation of the lesion.

Autoimmune-like tissue lesions. Autoimmune-like mononuclear cell infiltration in tissues was one of the changes observed in the various organs during the GvHR. In the liver and salivary glands of GvH-HLF1 mice mononuclear cells began to appear in the perivascular spaces and then in the periductal region of the bile duct in the liver or of the excretory duct of the salivary glands. Phenotyping of infiltrating lymphocytes revealed that not only CD4+ but also

Fig. 2. Macroscopic appearance of the kidney of chronically GvH-diseased mouse. Left, control mouse; Right, GvH-mouse at the 10th week. Whitish color of the kidney reflects anemia and nephrosclerosis, and residual ascites is seen in the hollow around the kidney of GvH-mouse.
CD8+ T cells were present in these lesions. Intraepithelial infiltration of T cells was observed, but the destruction of ducts was not always prominent. Class II MHC antigen was very clearly observed on the cell surface of the bile duct epithelium as early as the 3rd day of GvHR and the expression combined thereafter. It was expressed on the basolateral surface and sometimes in the cytoplasm of these cells, but the level of its expression varied among these cells. It seemed, however, that expression of Ia antigen on these cells was not enough to recruit activated lymphocytes to periductal regions or to destroy the ductal epithelium. This was because levels of Ia antigen expression of the ductal cells and periductal lymphocyte infiltration were not always parallel in the salivary gland. Moreover, it appeared that the environmental factor(s) was working in the processes that formed the lesions, because mice which were kept in conventional feeding conditions tended to have severer lesions than those kept in SPF conditions. Other investigators suggested organ specificity of lymphocytes infiltrating in the liver (Howell et al. 1989). It is possible that the organ specificity of infiltrating lymphocytes is produced by a microbial infection to a particular kind of cell, therefore the cells are specific to the microbial antigens in conjunction with either allogeneic class II antigens or syngeneic class II or I antigens expressed on the cells of the particular organ.

Other lesions of the GvHD. Huge splenomegaly weighing almost 1 g was observed in some mice around the 10th week of GvHR. Normal histological distinction of red and white pulps of the spleen disappeared in such mice and was replaced by uniform proliferation of lymphoblasts (Fig. 3), since more than 70% of cells in such spleens were medium- or large-sized T blasts. Infiltration of the same kind of cells into the liver, kidney and salivary glands was also observed, and the dense lymphoid infiltration partially destroyed specific cellular components of these organs. These histopathological changes might suggest this to be T cell malignancy, but the real nature, whether it was oligoclonal hyperplasia of alloantigen activated T lymphocyte or monoclonal and neoplastic proliferation of T cells, remains to be elucidated.

Another type of splenomegaly, though it was not as large as those explained above, was due to a prominent extramedullary hematopoiesis. Mature granulocytes and megakaryocytes proliferated in such spleen and replaced lymphoid components which otherwise were present in the white pulp (Fig. 3). It is apparent that these changes are among the features of GvHD and their expression could be attributable to the improper regulation of cellular proliferation and/or differentiation. However, it is not yet known which mechanisms trigger the proliferation of either T lymphoblasts or mature granulocytes and megakaryocytes.

Characteristic cellular events in D2-GvHR

Difference of cellular pattern between B6- and D2-GvHR. Both CD4+ and
CD8+ T cells were activated in B6- or D2-GvHR, because the whole MHC antigens were different between these two strains. However, the pattern of GvHR and therefore of GvHD was quite contrasting. Characteristic cellular aspects of the reactions were apparent in the ratio between CD4+ and CD8+ T cells and in the state of chimerism in the spleen (Fig. 4). In B6-GvHR, which showed symptoms of acute GvHD, CD8+ cells dominated over CD4+ cells after 2 weeks of GvHR. As a consequence the CD4/CD8 ratio became far below 1. In contrast to B6-GvHR, CD4+ cells were predominant throughout the D2-GvHR.

Different CD4/CD8 ratios in these GvHRs seemed to reflect the difference of the response of CD8+ and not of CD4+ T cells. The fraction of CD8+ cells in the spleen of the 2nd week of B6-GvHR was 75%, whereas that of D2-GvHR was 30%. In contrast, the percentage of CD4+ cells in the GvHR-spleen was not so different between the two. These differences might partly be explained by the different content of CD8+ cells in the grafts and their ability to respond to class I MHC antigens (Sprent and Schaefer 1989). In parallel with this notion, we observed that the suppressive syndrome in NZB→(NZB×NZW) F1, GvHR/D became milder if graft spleen cells from aged NZB were inoculated instead of those from younger mice. This can be ascribed to the age-associated and antibody-mediated decrease of CD8+ T cells in the NZB spleen.

Active participation of host CD8+ cells in D2-GvHR. The chimerism of graft and host cells in the GvHR-spleen was also quite contrasting between the two

Fig. 3. Histopathological appearance of the huge spleen of HLF1 mouse at the 13th week of GvHR. Left, predominant and uniform proliferation of blastic T cells. Distinction between the white and red pulps disappeared (original magnification ×50). Right, prominent extramedullary hematopoiesis. Megakaryocytes and mature granulocytes occupied the whole area of the spleen (×200).
GvHRs (Fig. 4). We considered that the two kinds of cellular aspects might be related to each other, because if we induced D2-GvHR in aged BDF1 mice, some mice, but not all, showed B6-GvHR-type reaction, i.e., both the CD8+ cell dominance and the graft cell dominant chimerism were observed at the same time. This is why we examined the kinetics of the response of CD8+ T cells, where we differentiated graft derived cells from the host ones. As shown in Fig. 5, graft derived CD8+ T cells transiently outnumbered those of host origin at days 5 and 7 of GvHR, but during the rest of the period of GvHR, host CD8+ were generally predominant. That the change of the number of these cells during D2-GvHR was not incidental but active was proved by an experiment in which host CD8+ cells were removed before the inoculation of D2 cells into BDF1. As shown in Table 3, chimerism in which graft cells were dominant was induced in CD8+ cell-deficient BDF1, and renal failure which was one of the clinical manifestations of chronic GvHD was not observed in such mice. This indicates that the host CD8+ T cells are a necessary component in D2-GvHR, because those cells prevent the massive penetration of graft cells in the spleen of D2-GvHR. This eventually makes the GvHR continue for a longer period.

Hybrid anti-parent response is known as a hybrid resistance to hemopoietic histocompatibility antigens. However, effector cells of this resistance is generally considered to be CD4-,CD8- NK cells (Yankelevich et al. 1989). Anti-parent (D2) activity by host (BDF1) CD8+ cells we observed might be directed to parent
CD8+ cells, because deletion of both the host and graft CD8+ cells after inoculation of D2 cells made the mice express class II-GvHR, but failed to establish the graft cell dominant chimism (Table 3). Hybrid anti-parent activity as observed by a different experimental parameter in D2-GvHR was reported to be

**Table 3. Active participation of host CD8+ cells in DBA/2→(C57BL/6×DBA/2) F1 GvH reaction**

<table>
<thead>
<tr>
<th>Treatment of thymectomized mouse</th>
<th>Timing</th>
<th>Fraction of graft cells in spleen</th>
<th>Clinical sign</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ICGN</td>
</tr>
<tr>
<td>no</td>
<td></td>
<td>29.5</td>
<td>++</td>
</tr>
<tr>
<td>Anti-L3T4</td>
<td>preGvHR</td>
<td>30.0</td>
<td>++</td>
</tr>
<tr>
<td>Anti-Lyt2</td>
<td>preGvHR</td>
<td>68.0</td>
<td>–</td>
</tr>
<tr>
<td>Anti-Lyt2</td>
<td>postGvHR</td>
<td>10.0</td>
<td>++</td>
</tr>
</tbody>
</table>

10×10^7 DBA/2 spleen cells were injected iv. into BDF1, and sacrificed at the 13th week of GvHR. Chimerism was analyzed flow cytometrically by FACStar, and H-2b negative cells were considered to be graft origin. Figures represent average of 3–4 mice and expressed as % of total spleen cells. Immune complex glomerulonephritis (ICGN) was examined by immunohistopathology and proteinuria was tested by Albstics.
controlled under multiple recessive genes of D2 (Ishikawa et al. 1984).

**Concluding remarks**

Experimental chronic GvHR/D using parent → F₁ system showed a variety of clinical symptoms. It is considered that these were induced by the abnormal immune regulation which occurred in the GvHR-mouse. The system is not necessarily a close model of human bone marrow transplantation, because GvHR was induced across the barrier of MHC antigens and in the intact animals. However, it is considered to be a good experimental model to analyze any disease occurring under immune dysregulation. We are currently studying the relationship between the cytokine molecules working in situ and cellular changes occurring in the chronically GvH-diseased mouse.

**References**


Chronic GvHR and GvHD


