THE DEVELOPMENT of two cellular techniques, the mixed lymphocyte culture and cell-mediated lympholysis, both now used throughout the world, made it possible to imitate human organ transplantation reactions in vitro. Obviously, these assays reflect only a specific aspect of the complex interactions involved in organ transplantation. Awareness of the limitations of in vitro observations is essential when in vivo situations are to be evaluated. The results of the in vitro studies presented here must be interpreted with these restrictions in mind.

The possible clinical relevance of in vitro analysis of cytotoxic T cell (CTL) activity in renal and bone marrow transplant recipients was evaluated.

In kidney transplantation, failure of a recipient's posttransplantation lymphocytes to elicit in vitro CTL responses against kidney donor splenocytes has been shown to correlate significantly with kidney allograft survival, as documented in several reports. The absence of host CTL directed specifically against the graft histocompatibility antigens has been observed not only at the effector cell population level. Frequency analyses of alloreactive CTL precursors (CTL-p) in a group of kidney recipients demonstrated a decrease in donor-specific CTL-p frequency after transplantation, whereas the frequency of irrelevant third-party donor-reactive CTL-p remained unchanged. Thus, it appears that a marked decrease in the number of in vitro donor-directed CTL can coincide with in vivo graft tolerance. Functional in vitro clonal deletion can, however, be compensated by the addition of exogenous IL2. It is likely that this balance can be disturbed by activation of the immune system, for example by viral infection. This hypothesis is supported by the observations of Grundy and Shearer, who reported that in certain strains of mice an immunoenhancing effect of the host immune response to foreign MHC antigens occurred during murine cytomegalovirus infection. Moreover, an increment in the number of IL2 receptor expressing cells at the peak of inflammation has also been described.

It is evident that the state of acquired in vivo immunologic tolerance as reflected by in vitro kidney donor-specific CTL nonresponsiveness is the ultimate goal of transplantation immunologists. What, however, is the significance of this goal? Do the patients who display long term kidney donor-specific CTL nonresponsiveness suffer a disadvantage? The increased incidence of malignant tumors among organ recipients, as observed in the past decade, has been attributed to immunosuppressive therapy and its effects, but it might also be a direct consequence of the state of acquired tolerance. In support of the latter hypothesis are experimental findings, described previously, that showed the presence of "linked nonresponsiveness" after renal transplantation: lymphocytes from renal allo-

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grafted patients with a well functioning graft display kidney donor-specific CTL nonresponsiveness in vitro. In addition, these lymphocytes do not exhibit a cytolytic response upon stimulation with cells from unrelated blood donors selected for the presence of kidney donor HLA B locus antigens. Moreover, cells from panel members matched to the kidney donor at the HLA-B locus but mismatched at the A locus suppressed CTL activity against any HLA A antigen presented on the same stimulator/target cell (Table 1).

If tolerance for donor specific HLA B locus alloantigens is acquired and consequently the "linked no 1-responsiveness" becomes manifest, then the immunologic tolerance might be much broader than anticipated. The biologic relevance of these phenomena with respect to tumor evolution after renal transplantation has still to be demonstrated.

CTI activity in bone marrow transplantation was also investigated and the clinical relevance of in vitro CTI activity on the development of graft-vs-host disease (GVHD) was evaluated. Previously we reported the presence of CTI activity in recipients of an HLA genetically identical bone marrow graft. As yet anti-host CTL activity of posttransplant peripheral blood lymphocytes (PBL) could be demonstrated mainly (but not exclusively) in patients suffering from chronic GVHD but not in patients without GVHD 15. Some of these CTI populations were subsequently analyzed and found to be directed against minor histocompatibility (minor H) antigens requiring self HLA class I antigens for recognition. Analysis at the population level revealed relatively high phenotype frequencies for the minor H antigens (provisional designation HA-1 to HA-5) identified. Limited family studies showed a Mendelian mode of inheritance of these antigens. The possible relevance of minor H antigens to the development of GVHD was investigated by retrospective typing analysis of a series of HLA-identical bone marrow donor/recipient combinations. To date, the results of this analysis indicate that incompatibility for one (or more) minor H antigen between HLA-identical bone marrow donor and recipient occurred predominantly in the group of patients suffering from chronic GVHD 16. In summary, the facts that minor H antigen-specific CTI are generated from PBL in patients with chronic GVHD and that mismatches of one of the HA antigens occur in patients who suffer from chronic GVHD not only indicate the relationship between the in vitro observations and the clinically manifested GVHD, but also support the hypothesis that host-directed minor H antigen-specific CTI play a role in the development of GVHD. The important question is, of course, do these patients benefit from antihost CTI activity? The hypothesis that post bone marrow transplant antihost CTI activity may have a beneficial effect is based on the assumption that the postulated antileukemic potential is a desired side effect of the post bone marrow transplant complication GVH.

At present, extensive immunogenetic analyses and tissue distribution studies are in progress in an attempt to gain information about the most common (ie, most immunogenic) human minor H antigens and to determine their role in the pathogenesis of GVHD as well as their possible relevance in the graft-vs-leukemia (GVL) reaction. Hopefully such studies will facilitate the search for the exact balance between GVH and GVL, yielding a higher efficacy for clinical bone marrow transplantation.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Role of Kidney Donor HLA B Locus Antigens in Posttransplant Cytolytic Nonresponsiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unrelated Blood Donors With Kidney Donor Antigens</td>
<td>Cytolytic Response</td>
</tr>
<tr>
<td>HLA B (+ C) but not A</td>
<td>no</td>
</tr>
<tr>
<td>HLA A (+ C) but not B</td>
<td>yes</td>
</tr>
</tbody>
</table>

*Posttransplant peripheral blood lymphocytes from CML nonresponsive recipients were stimulated in vitro with either kidney donor HLA B (and C) or kidney donor HLA A (and C) antigens presented on lymphocytes of unrelated blood donors.
† Any foreign HLA A locus antigen.
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REFERENCES

2. Hayry P, DeFeo V. Science 168 133, 1970
9. Grundy H. Shearer GM. Transplantation 37 484, 1984
14. Goulmy E. Prog Allergy 36 44, 1985