Antigen Presentation by Human Antigen-Presenting Cells to Antigen-Specific Xenogeneic Murine T Cells

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Abstract  Successful antigen presentation by xenogeneic human antigen-presenting cells (APC) to stimulate the proliferation of antigen-specific, keyhole limpet hemocyanin (KLH)-specific, ovalbumin (OVA)-specific, and purified protein derivative of Mycobacterium tuberculosis (PPD)-specific murine T cells was observed. Evidence indicating a direct cell interaction between antigen-specific murine T cells and xenogeneic human APC was given by experiments using antigen-specific murine T cell clones. The OVA-specific B10.S(9R) T cell line (9-0-A1) and PPD-specific B10.A(4R) T cell line (4-P-1) were stimulated by both xenogeneic human APC and murine APC from syngeneic or I-A compatible strains, while the PPD-specific human T cell line (Y-P-5) was stimulated by autologous human APC but not by murine APC. Anti-HLA-DR monoclonal antibodies (MoAb) blocked the xenogeneic human APC-antigen-specific murine T cell clone interaction. Thus, human xenogeneic APC can stimulate antigen-specific murine T cells through HLA-DR molecules in the same manner as syngeneic murine APC do through Ia molecules coded for by the I region of the H-2 complex, while murine APC failed to present antigen to stimulate human antigen-specific T cells.

The polymorphism of the major histocompatibility complex (MHC) coded for by large numbers of alleles has been thought to function for immune recognition of self or non-self elements by T and B cells. This polymorphism apparently has an important role in T cell activation by antigen-presenting cells (APC) and in genetically restricted cell interactions between APC, T cells and B cells in various rodents (2, 3, 18, 22, 24, 30, 31, 33). Recently, new critical techniques for the study of the immune system have been developed, such as the establishment of hybridomas secreting monoclonal antibodies (MoAb) and of T cell clones. These techniques have overcome the difficulties in analyzing immune responses complicated by the existence of huge numbers of antigen-specific T and B cell clones and of complex

Abbreviations: APC, antigen-presenting cells; CTL, cytotoxic T lymphocytes; GAT, synthetic polymer Glu^{60}Ala^{30}Tyr^{10}; KLH, keyhole limpet hemocyanin; LNL, lymph-node lymphocytes; MHC, major histocompatibility complex; MLR, mixed lymphocyte reaction; MoAb, monoclonal antibodies; OVA, ovalbumin; PBL, peripheral blood leukocytes; PETLES, peritoneal exudate T-lymphocyte-enriched cells; PPD, purified protein derivative of Mycobacterium tuberculosis.
sets of interacting lymphoid cells and antigen-presenting cells. The application of these techniques has revealed the detailed structures and regulatory mechanisms of the immune system at the molecular and cell clone level. However, some results inconsistent with previous reports regarding the role of the polymorphism of the MHC in T cell activation were observed when such recently developed techniques were introduced.

By using monoclonal antibodies, Lunney et al have revealed cross-reactivity between murine and human non-polymorphic determinants of class II antigens (12). Similar serological interspecies cross-reactivities of the MHC were later reported by many other investigators. Pierres et al reported serological cross-reactivity of murine polymorphic determinants with either human polymorphic or shared determinants (16). In addition, Longo and Schwartz reported that the ovalbumin (OVA)-specific B10.A murine T cell clone is cross-reactive with allo-Ia antigens (I-A$^d$) (11). A number of antigen-specific proliferative T cell clones have been established that both recognize nominal antigens (e.g. synthetic polymer Glu$^{60}$ Ala$^{30}$ Tyr$^{10}$ (GAT), cytochrome c, etc.) in the context of self and cross-reactive allo-Ia determinants and are allo-Ia reactive (13, 14).

Furthermore, several groups including our own have reported interspecies cross-reactivity of the MHC recognition of T cells in the mixed lymphocyte reaction (MLR) and cytotoxic T lymphocyte (CTL) responses (6, 10, 23, 32). We recently reported on the phylogenetic hierarchy of the Ia function in terms of antigen presentation by antigen-presenting cells to stimulate antigen-specific T cell responses. Murine APC from various strains which possess a variety of cross-reactive polymorphic determinants with human class II antigens failed to present the antigen, purified protein derivative of Mycobacterium tuberculosis (PPD), to human T cells (29). On the other hand, in this paper we present data showing that mouse antigen-specific T cell clones as well as bulk populations of antigen-primed murine T cells were stimulated with antigen presented by xenogeneic human APC and murine syngeneic APC. Thus, evidence indicating direct cell interaction between antigen-specific murine T cells and xenogeneic human APC was given. Furthermore, the MT-3 determinant of the HLA-DR molecule on human APC was shown to function for the stimulation of antigen-specific, I-A restricted murine T cell clones.

MATERIALS AND METHODS

Animals. B10.S(9R)/Sn, B10.S/Sn, and B10.A(4R)/Sn mice were bred and maintained in our laboratory. B10.A/Sn SLC and C57BL/6 CrSlc (B6) were purchased from Shizuoka Laboratory Animal Center (Hamamatsu). Mice of both sexes of between 6 and 18 weeks of age were used in this study.

Antigen and immunization. Ovalbumin (OVA: Sigma Chemical Co., St. Louis Mo.) and keyhole limpet hemocyanin (KLH: Schwarz/Mann, Orangeberg, N.Y.) were emulsified in complete Freund's adjuvant containing, 1 mg/ml of killed M. tuberculosis (Aoyama B) organisms (Nippon BCG Co., Tokyo). PPD was purchased from Nippon BCG Co. Mice were immunized by injection of 50 μg of antigen in a
total volume of 0.1 ml of emulsion into the hind footpads, as previously described (19–21, 31).

Cell preparation. The original and slightly modified procedures for preparing peritoneal exudate T-lymphocyte-enriched cells (PETLES) and lymph-node lymphocytes (LNL) are described in detail elsewhere (5, 19–21). Human peripheral blood leukocytes (PBL) were separated from heparinized peripheral venous blood from donors by Ficoll-Conray gradient centrifugation as described in detail previously (15, 25). In order to eliminate murine and human APC, B10.S(9R) PETLES or LNL and human PBL were treated with monoclonal ISCR3 antibody (anti-I-Ek) and monoclonal anti-HLA-DR (clone L243) and rabbit complement as previously described (27, 29, 32).

Monoclonal antibodies (MoAb). The monoclonal anti-I-Ek antibody (ISCR3) cross-reactive with HLA-DR antigen was kindly provided by Dr. Nobukata Shinohara (National Cancer Institute, NIH, Bethesda, Md.) (27). The anti-I-Ak MoAb (IAK1) was prepared as described previously (32). Anti-HLA-DR (clone L243) MoAb was purchased from Becton Dickinson (Sunnyvale, Calif.).

Cell culture and murine T cell proliferative responses to antigen-pulsed syngeneic APC or xenogeneic human APC. The procedure for antigen-pulsing to murine and human APC, and the proliferative response assay were carried out as described in detail previously (5, 25, 29, 31, 32). According to the results of preliminary experiments to determine the optimal conditions for obtaining maximum response, 1 × 10^5 murine spleen cells and 1–3 × 10^4 human PBL were used as APC sources. In vitro primary responses were measured on day 5, and the responses of the T cell line were measured on day 3 of the assay culture in round-bottomed microtiter plates (1-63302 Nunclon, Nunc, Roskilde, Denmark). The procedures for culturing the cells and measuring the responses have been described (1, 15, 25, 29, 32). The results were expressed either as mean counts per min (cpm) ± the standard error of the mean (SEM), or as the difference between antigen-stimulated and control responses (Δ cpm).

Preparation of antigen-specific T cell lines. Seven to 8 days after antigen priming, T cells from draining lymph nodes were prepared by passage over nylon wool columns, and were cultured in RPMI 1640 medium containing 10% fetal calf serum, plus irradiated (2,000 rad) antigen-pulsed APC. Antigen-specific T cell lines were established by serial restimulation of lymph node-derived T cells or human PBL in vitro by antigen-pulsed APC and the method of limiting dilution described elsewhere (8). The T cell lines 9-0-A1, 4-P-1, and Y-P-5 were derived from OVA-primed B10.S(9R), PPD-primed B10.A(4R), and tuberculin reaction-positive human YA (A2, AW31, BW35, BW59, C1, C4, DR4, MT3) PBL, respectively.

Blocking assay. In order to determine the inhibitory effects of the anti-HLA-DR and IAK1 MoAbs on the proliferative responses of murine T cells to murine or human APC pulsed with antigen, various doses of the antibodies were added to the cultures in KLH-, OVA- or PPD-specific proliferative assays. The blocking effect was calculated by the following formula:
% blocking = \left(1 - \frac{\text{antigen-specific response with antibody (cpm)}}{\text{antigen-specific response without antibody (cpm)}}\right) \times 100.

**HLA phenotype.** HLA-A, -B, -C, and -DR phenotypes of human PBL were established by standard serologic techniques in Sakura National Hospital (Sakura, Chiba). HLA-MT phenotypes were determined by Bousei Science Co. (Kanagawa).

## RESULTS

### Hierarchy of Antigen-Presenting Ability of Human APC and Murine APC in Xenogeneic APC-T Cell Interactions

An earlier study demonstrated that antigen-specific murine T cells are stimulated by not only syngeneic murine APC but also xenogeneic human APC, whereas murine spleen cells from various H-2 congenic mice failed to stimulate antigen-specific human T cells (29). Thus, a hierarchy of antigen-presenting ability in the xenogeneic human and mouse APC-T cell interaction was shown to exist. The specificity of antigen presentation by human APC for stimulation of antigen-primed mouse T cells was antigen specific as shown in Table 1. KLH-primed B10.S(9R) LNL were responsive to both KLH-pulsed B10.S(9R) APC and KLH-pulsed human APC. However, when KLH-primed T cells were cultured with human APC pulsed with antigen unrelated to the priming antigen, such as PPD, the response was not different from the control response with antigen-non-pulsed APC. Similarly,

<table>
<thead>
<tr>
<th>APC source</th>
<th>KLH-primed</th>
<th>PPD-primed</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>cpm ± SEM</td>
<td>( \Delta )cpm</td>
</tr>
<tr>
<td>B10.S(9R) spleen cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-pulsed</td>
<td>1,402 ± 36</td>
<td>1,379 ± 15</td>
</tr>
<tr>
<td>KLH-pulsed</td>
<td>35,353 ± 1,435</td>
<td>33,951</td>
</tr>
<tr>
<td>PPD-pulsed</td>
<td>1,436 ± 89</td>
<td>34</td>
</tr>
<tr>
<td>Human PBL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-pulsed</td>
<td>9,361 ± 543</td>
<td>12,917 ± 2,560</td>
</tr>
<tr>
<td>KLH-pulsed</td>
<td>35,769 ± 4,054</td>
<td>26,408</td>
</tr>
<tr>
<td>PPD-pulsed</td>
<td>12,709 ± 391</td>
<td>3,348</td>
</tr>
</tbody>
</table>

\( ^{a)} \) KLH- or PPD-primed B10.S(9R) LNL (3 \times 10^4 cells per well) were pre-treated with anti-Ia Ab (ISCR3) plus C as described in "MATERIALS AND METHODS." The responses of APC-depleted KLH-primed B10.S(9R) LNL to medium, KLH (20 μg/ml), and PPD (5 μg/ml) were 669 cpm, 1,163 cpm, and 690 cpm, respectively. The responses of PPD-primed cells to medium, KLH and PPD were 619 cpm, 1,020 cpm, and 507 cpm, respectively.

\( ^{b)} \) The APC-depleted cells were cultured with either antigen-non-pulsed or antigen KLH- or PPD-pulsed B10.S(9R) spleen cells (1 \times 10^5) or human PBL (3 \times 10^4) as APC sources after inactivation by X-ray irradiation.
PPD-pulsed human APC stimulated PPD-primed B10.S(9R) T cells but not KLH-primed T cells. Antigen-specific murine proliferative cells responsive to antigen-pulsed xenogeneic human APC were Ia−, Thy 1+ and Lyt 1+2− cells (data not shown).

**Blocking Effects of Anti-Human Class II Antibodies on the Cell Interaction between Antigen-Specific Mouse Proliferative T Cells and Human APC**

As previously reported, class II molecules such as HLA-DP, -DQ and -DR molecules are involved in the allogeneic and xenogeneic MLR responses and antigen-specific and mitogen-induced human proliferative T cell responses (3, 9, 15, 23, 25, 32). To establish whether or not the functional molecule on human APC for stimulation of antigen-specific mouse T cell proliferation is the HLA-DR molecule, we performed blocking experiments using anti-HLA-DR MoAb (L243) and an antigen-specific murine T cell line. As depicted in Fig. 1a, the antigen-specific proliferative responses of the PPD-specific murine T cell line 4-P-1 to antigen-pulsed human APC were significantly inhibited by the anti-HLA-DR MoAb. A dose-dependent blocking effect was clearly observed. Antigen presentation to the cell line by syngeneic B10.A(4R) APC was blocked by the anti-I-Ak MoAb (IAK1) but not by the anti-HLA-DR MoAb. Thus, xenogeneic human APC presented the antigen with the HLA-DR molecule to the murine T cells. No xenogeneic cell interaction between PPD-pulsed B10.A(4R) APC and PPD-specific human T cell line Y-P-5 was observed (Fig. 1b). These data again show that a phylogenetic hierarchy of antigen-presenting ability exists in xenogeneic cell interaction, that is, the antigen-presenting ability of human APC is dominant over that of mouse APC for stimulation of antigen-specific T cell clones.

**Antigen Presentation by Xenogeneic Human APC to OVA-Specific, I-A Restricted B10.S(9R) T Cell Line 9-0-A1**

In the next experiments, the specificity of class II antigen recognition of antigen-specific murine T cells was analyzed by using the OVA-specific T cell line. The frequency of T cell lines reactive with antigen-pulsed human APC varied from less than 0.5% to 3% of that of T cell lines reactive with antigen-pulsed syngeneic APC. As shown in Table 2, one of the OVA-specific and I-A restricted B10.S(9R) T cell lines, 9-0-A1, responded to xenogeneic human APC as well as to syngeneic B10.S(9R) APC. The cell line was stimulated by xenogeneic human YM APC (12,905 ± cpm) and human YUK APC (6,315 ± cpm), but not by human YAK APC (<0 ± cpm). Human YM and YUK shared the MT3 antigen but no other determinants of the HLA-DR molecule, while human YM and YAK shared the DR2 and MT1 antigens. Thus, MT3 may have a critical role in stimulating the proliferative response of OVA-specific B10.S(9R) T cell line 9-0-A1.

**Genetic Analysis of Antigen Presentation by Xenogeneic Human APC to KLH-Specific B10.S (9R) T Cells**

In the next experiment, genetic analyses of the xenogeneic cell interaction be-
Fig. 1. Blocking effect of anti-HLA-DR MoAb on human APC-murine T cell xenogeneic interaction. PPD-specific B10.A(4R) T cell line 4-P-1 cells (1 × 10⁴) and PPD-specific human T cell line Y-P-5 cells (3 × 10⁴) were cultured with 3 × 10⁴ Human YA APC pulsed with PPD (ΔΔ) or non-pulsed (□□□□). Various concentrations of anti-HLA-DR MoAb (clone L243) and anti-I-Ak MoAb (IAK1) were added to the cultures. The responses were assessed 3 days later by measuring the incorporation of a 20-hr pulse of [³H]Tdr. The results are expressed as cpm, and the vertical bars show the standard error of the mean. The HLA phenotypes of Human YA were HLA-A2, AW31, BW35, BW59, C1, C4, DR4, and MT3. The responses of 4-P-1 cells to PPD (2 μg/ml) and medium alone were 1,177 ± 168 cpm and 822 ± 135 cpm, respectively in the absence of APC. The responses of Y-P-5 cells to PPD (2 μg/ml) and medium alone were 1,891 ± 1,137 cpm and 1,056 ± 197 cpm, respectively, in the absence of APC. No significant proliferative responses of 4-P-1 cells and Y-P-5 cells were observed to other antigens, such as OVA and KLH.
human APC and murine T cells were performed by using another antigen, KLH-specific murine T cells. As shown in Table 3, KLH-specific B10.S(9R) T cells were stimulated by human AF, YA, and YUK, but not by human YAK and MA. Human AF, YA, and YUK shared DR4 and MT3, while human YAK and MA possessed neither DR4 nor MT3. Thus, the MT3 determinant seems to regulate the cell interaction between KLH-specific B10.S(9R) T cells and human APC, although the participation of the DR4 determinant for stimulation of murine T cells has not been elucidated.

**DISCUSSION**

Lindahl and Bach (10) and Swain et al (23) have shown that human lymphocytes recognize the polymorphic determinants of murine H-2 (mainly I-A subregion) in the human anti-mouse MLR. However, as previously reported, human antigen-specific T cells cannot be stimulated by antigen-pulsed murine APC (29), although large numbers of Ia determinants shared by humans and mice have been identified (1, 12, 15–17, 25). In this study, we demonstrated xenogeneic cell interaction between human APC and antigen-specific murine proliferative T cells. These data show that there is a hierarchy of antigen presentation between human and
Table 3. Genetic analysis of antigen presentation by xenogeneic human APC to KLH-specific B10.S(9R) T cells

<table>
<thead>
<tr>
<th>Antigen-presenting cells</th>
<th>HLA-phenotype&lt;sup&gt;b&lt;/sup&gt;</th>
<th>KLH-specific B10.S(9R) T cell&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Non-pulsed (cpm ± SEM)</td>
</tr>
<tr>
<td>Syngeneic B10.S(9R) spleen cells</td>
<td></td>
<td>7,539 ± 3,175</td>
</tr>
<tr>
<td>Xenogeneic human PBL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 YAK AW24 BW52</td>
<td>DR2, MT1</td>
<td>7,208 ± 1,134</td>
</tr>
<tr>
<td>2 AF A2, A11 BW51, BW55 CW1</td>
<td>DR4, MT3</td>
<td>12,541 ± 2,118</td>
</tr>
<tr>
<td>3 YA A2, AW31 BW35, BW59 C1, C4</td>
<td>DR4, MT3</td>
<td>10,975 ± 737</td>
</tr>
<tr>
<td>4 YUK A11, AW24 B15, BW51 CW4</td>
<td>DR4, MT3</td>
<td>7,269 ± 874</td>
</tr>
<tr>
<td>5 MA A33 B12</td>
<td>DR1, DR6, MT1, MT2</td>
<td>12,514 ± 366</td>
</tr>
</tbody>
</table>

<sup>a</sup> KLH-specific B10.S(9R) were treated with anti-Ia antibody plus C to remove syngeneic APC. After the treatment, the number of living cells was adjusted to 1 × 10⁶ per well and the cells were cultured with KLH-pulsed or non-pulsed, 1 × 10⁵ B10.S(9R) spleen cells or 3 × 10⁴ human PBL.

<sup>b</sup> HLA-A, -B, -C, and -DR phenotypes of human PBL were established by standard serologic techniques.
mouse APC to stimulate antigen-specific proliferating T cells; that is, human APC can present antigens to murine T cells, but murine APC fails to present antigens to human T cells. Furthermore, we observed similar phylogenetic hierarchy of antigen-presenting ability in xenogeneic mouse, rat and human T cell interactions (manuscript in preparation); that is, the antigen-presenting ability of human APC is dominant over that of rat (Sprague-Dawley, SD) APC and the ability of SD APC is dominant over that of mouse (B10.S(9R)) APC.

The evidence that class II molecules on human APC have a critical role in the antigen presentation to murine T cells was provided by blocking experiments using anti-human class II antibodies. Antigen presentation by human APC to murine T cells was blocked by the anti-HLA-DR MoAb (Fig. 1). So far, at least three distinct human class II antigens (HLA-DR, -SB, -MB/MT/DC or more recently HLA-DR, -DP, -DQ) have been identified (4). The involvement of other human class II antigens, such as HLA-DP and -DQ antigens, in the xenogeneic human APC-murine T cell interaction remains to be clarified. In our experiments, anti-human-DC MoAb (anti-Leu 10 MoAb) (26) completely failed to block the xenogeneic human APC and murine T cell interaction (data not shown). The evidence of direct cell interaction between murine T cells and human APC was shown by the experiments in which an OVA-specific B10.S(9R) T cell line (9-0-A1) and a PPD-specific B10.A(4R) T cell line (4-P-1) could be stimulated by human APC as well as by syngeneic murine APC as shown in Table 2 and Fig. 1a. In addition, the proliferative response of the PPD-specific B10.A(4R) T cell line, 4-P-1, stimulated by syngeneic APC, was blocked by the anti-I-Ak MoAb (IAK1) (Fig. 1a). Thus, the antigen-specific murine T cells triggered by xenogeneic human APC are the same T cell population as triggered by syngeneic murine APC, and the cells respond to the shared determinants of human DR antigen with murine allele-specific determinants. Recently, Hurley et al reported that the majority of the population of molecules bearing MT2 and MT3 determinants may be DR molecules (7). More recently, MT2 and MT3 were renamed DRW52 and DRW53, respectively (4). One of the possible determinants on the DR molecule functioning in the xenogeneic APC-T cell interaction may be the MT3 determinant as shown in Tables 2 and 3. Waters et al also suggested that the KLH-specific I-E-restricted murine T cell hybridoma was stimulated by human APC possessing the MT3 determinant, although they failed to block IL-2 production of the hybridoma stimulated by human APC with the use of anti-MT3 MoAb (28).

Matis et al (13) and Naquet et al (14) reported that some T cell clones specific for the antigens cytochrome e or GAT, were stimulated by not only syngeneic or I-region compatible murine APC but also allogeneic APC together with primed antigens (cross-presentation) or without antigens (allo-reactive). One of the possible mechanisms of the xenogeneic human APC and murine T cell interaction is that some antigen-specific murine T cell clones are cross-stimulated by antigen-pulsed xenogeneic human APC in a fashion similar to cross-stimulation by allogeneic murine APC in the context of the primed antigen. As reported by Lunney et al (12), Pierres et al (16), and others (1, 27), interspecies cross-reactive determinants of class
II molecules exist as either polymorphic (varying from broad to narrow) or non-polymorphic determinants defined by antibodies or recognition by B cells. Thus, it has been demonstrated both serologically and functionally (as in T cell activation) that interspecies- and intraspecies-crossreactivities of class II molecules exist. Another important finding in this study is the existence of a hierarchy of human and mouse APC in antigen presentation for antigen-specific T cell stimulation. The failure of mouse APC to stimulate human T cells is not due to the kinetic difference and the presence of suppressor cells shown in the previous paper (29). The reason for this phenomenon might be either that murine Ia epitopes of Ia antigen do not have the triggering ability equivalent to that of human DR determinants or the molecule necessary for stimulation of antigen-specific human T cells, or that human beings lack T cell clones cross-reactive with murine Ia molecules in the context of nominal antigens.

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