Brief Definitive Report

Donor Major Histocompatibility Complex (MHC) Peptides Are Presented by Recipient MHC Molecules during Graft Rejection

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Summary

Peptides from donor major histocompatibility complex (MHC) molecules were examined for their activation of allogeneically primed T cells. After immunization with either allogeneic spleen cells or a skin allograft, primed T cells proliferate in response to peptides derived from polymorphic regions of α and β chains of class II allo-MHC molecules. The results demonstrate that presentation of donor-MHC peptides by host-derived antigen-presenting cells is a common event in vivo. Thus, self-restricted T cell recognition of processed alloantigens may play a critical role in transplantation. An in-depth understanding of this response may result in the development of additional molecular therapies to combat allograft rejection.

During transplantation or allograft rejection, determinants encoded within the MHC of the donor are recognized by T lymphocytes of the recipient. This recognition results in a potent immunological reaction in which donor cells are rapidly and specifically killed, and the graft is destroyed (1, 2). It is generally accepted that T cells recognize foreign antigens in the form of peptides presented in association with self-MHC molecules. However, the molecular basis for the recognition of the allogeneic target is still controversial. Three nonexclusive models for the target structure that is recognized by alloreactive T lymphocytes have been proposed: (*a*) alloreactive T cells recognize polymorphic motifs present on the intact allo-MHC molecule, regardless of peptides bound to them; (*b*) self- (recipient) or allo- (donor) derived peptides interact with the native allo-MHC molecules to create a series of new determinants recognized by T cells; (*c*) the allo-MHC molecules are processed into peptides and presented by self-MHC molecules to specific T cells (3, 4). In this report, we provide evidence for the third alternative.

Materials and Methods

Peptides. Peptides were synthesized in the Norris Cancer Center Microchemistry Laboratory (USC) with an automated peptide synthesizer (430A; Applied Biosystems Inc., Foster City, CA) using modified Merrifield chemistry. These peptides were also used in the studies reported in reference 5.

T Cell Proliferation. B10.A (H-2^a), BALB/c (H-2^d), and SJL/J (H-2^s) mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and bred at UCLA. BALB/c and SJL/J mice were immunized in the foot pads with 2×10^7 irradiated splenocytes in 25 μl, along with 25 μl of CFA (Difco Laboratories, Detroit, MI) on the dorsal surface of the foot. Popliteal lymph node cells and splenocytes were obtained 10 d later, and used in antigen-induced proliferation assays. Cell suspensions were cultured in 0.2 ml of serum-free HL-1 medium (Ventrex, Portland, ME), containing 2 mM glutamine in 96-well plates for 4 d (5×10^5 cells/well). Proliferation was assessed by the incorporation of 1 μCi [^{3}H]thymidine during the last 18 h of culture.

Skin Grafts. SJL/J and BALB/c mice were anesthetized, and then engrafted on the left side of the back with back skin (1-cm disk) from B10.A mice, according to the technique described by Billingham and Medawar (6).

Results and Discussion

The molecular identity of the target structure recognized by T cells during an in vivo alloresponse leading to graft rejection is obscure. To address this issue, BALB/c (H-2^d) and SJL/J (H-2^s) mice were primed with B10.A (A^k, E^k)-irradiated splenocytes. Lymph node T cell proliferation was tested 10 d later in response to peptides from polymorphic regions of the α and β chains of the A^k class II molecule. As shown in Fig. 1 *A*, three MHC class II peptides, A^k_α1-18, A^k_α68-81, and A^k_β1-16, elicited strong and dose-dependent T cell proliferation in SJL mice while two peptides, A^k_α49-63 and A^k_β58-71, were nonstimulatory. In contrast, in cultures from BALB/c mice primed with B10.A-irradiated splenocytes, only peptides A^k_β1-16 and A^k_β58-71 induced in vitro T cell proliferative responses (Fig. 1 *B*). It is of interest that two of these peptides, A^k_α68-81 and A^k_β58-71, cannot bind to B10.A

J. Exp. Med. © The Rockefeller University Press • 0022-1007/92/01/0305/04 $2.00
Volume 175 January 1992 305–308

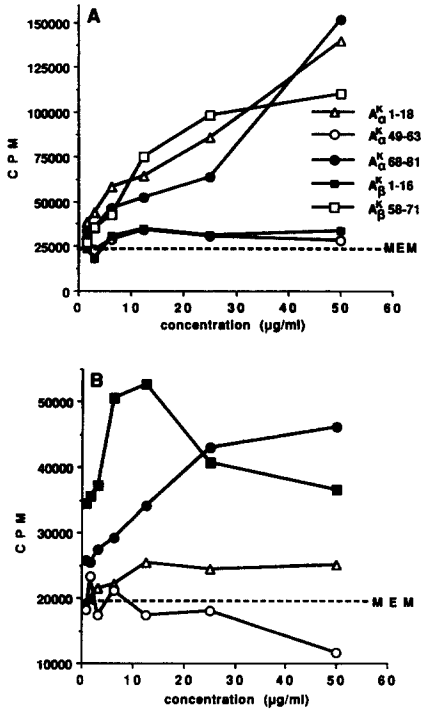

Figure 1. Recipient lymph node T cells proliferate to donor MHC peptides after immunization with allogeneic cells. Results are expressed as counts per minute obtained with lymph node cells stimulated in vitro with MHC peptides. Each group represents three SJL/J (*A*) and three BALB/c (*B*) mice tested individually. The positive control responses to purified protein derivative ranged from 152,000 to 197,420 cpm.

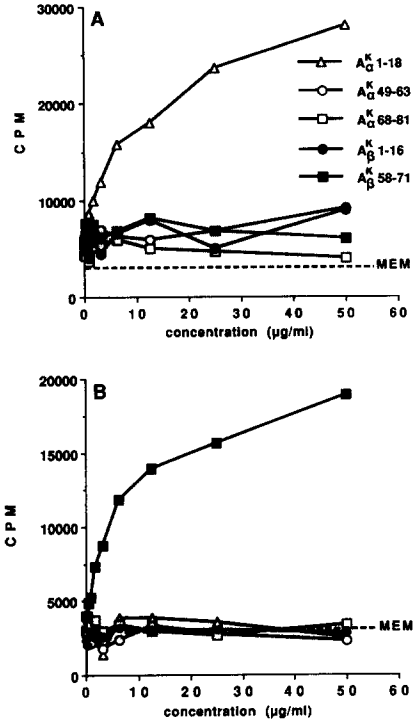

Figure 2. Recipient splenic T cells proliferate to donor MHC peptides during graft rejection. Results are expressed as counts per minute obtained with spleen cells stimulated in vitro with MHC peptides. Each group represents two SJL/J (*A*) and two BALB/c (*B*) mice tested individually.

MHC molecules, thereby eliminating their presentation by allogeneic MHC (5). Irrelevant A^s peptides corresponding to the same regions of the Ia molecule were inactive (data not shown). Lymph node cells from nonimmunized BALB/c or SJL/J mice or from B10.A mice primed with B10.A syngeneic irradiated splenocytes did not respond to any of the A^k-derived peptides (data not shown).

To determine whether analogous responsiveness to allo-MHC peptides is induced during allograft rejection, BALB/c and SJL mice were grafted with skin from B10.A animals. 10 d after the transplantation, splenocytes of the recipients were tested for in vitro proliferation to the A^k peptides. As shown in Fig. 2, SJL/J mice responded to A^k_α1-18 while BALB/c mice responded to A^k_β58-71, as was observed for immunizations performed with A^k-expressing allogeneic cells.

Table 1. *Immunization with donor MHC Peptides Reveals New T Cell Determinants*

	Recall with MHC peptides in vitro					
Priming with B10.A	No Peptide	A^k_α1-18	A^k_α49-63	A^k_α68-81	A^k_β1-16	A^k_β58-71
SJL/J						
Skin graft	5 ± 2	28 ± 8	7 ± 2	7 ± 2	9 ± 3	6 ± 1
Spleen cells	24 ± 3	139 ± 10	28 ± 4	109 ± 18	152 ± 21	33 ± 6
MHC peptide	5 ± 3	82 ± 5	152 ± 10	133 ± 8	103 ± 10	7 ± 2
BALB/c						
Skin graft	2 ± 1	2 ± 1	2 ± 0.8	ND	3 ± 2	19 ± 2
Spleen cells	19 ± 2	25 ± 6	21 ± 6	ND	49 ± 9	53 ± 6
MHC peptide	4 ± 3	3 ± 1	2 ± 0.5	ND	52 ± 5	89 ± 11

Results are expressed as counts per minute. The values that are significantly over the background counts ± SD are underlined.

It is notable that A^k_β1-16 and A^k_α68-81 no longer triggered T cell responses in SJL/J and BALB/c, respectively, after skin grafting, in contrast to splenocyte injection. Spleen cells from normal untreated mice and from B10.A mice that received an autograft with B10.A skin did not proliferate in response to the same class II MHC peptides (data not shown).

Direct immunization of BALB/c and SJL mice with the A^k-derived peptides resulted in T cell proliferation to the same determinants as after immunization with allogeneic cells. Moreover, this approach revealed an additional determinant on the A^k molecule that was undetected after immunization with allogeneic cells (Table 1). A^k_α49-63 was clearly immunogenic in SJL mice but could not elicit a T cell response in mice primed with splenocytes or with a skin graft. This peptide can be considered to contain a nondominant or cryptic determinant that is not produced efficiently during processing. In this sense, MHC molecules are similar to other protein antigens (e.g., lysozyme) which contain both dominant and cryptic determinant regions (7). It is noteworthy that upon skin grafting, only one of the four, or one of the two, potentially immunogenic peptides is presented in SJL/J and BALB/c, respectively; presumably, these are the most dominant determinants. This result indicates that the method of immunization influences the nature of the determinants recognized by T cells on a multideterminant antigen and suggests that during the process of skin graft rejection, because of less efficient processing, only the predominant determinants will be presented to induce T cell proliferation.

The ability to recall responses with peptides in vitro after in vivo priming with intact cells or with skin grafting clearly indicates that donor MHC class II processing and peptide presentation occur during the course of an in vivo allogeneic response. The proliferative responses to the two peptides A^k_α68-81 and A^k_β58-71, which failed to bind to donor A^k or E^k MHC molecules, must have been presented by the recipient ($H\text{-}2^s$ or $H\text{-}2^d$) MHC molecules. This demonstrates that one portion of the in vivo alloresponse to these allo-MHC peptides is restricted to the MHC molecules of the recipient. In the case of A^k_β1-16, we cannot exclude the possibility that this MHC class II peptide was presented in association with the donor-derived class II MHC molecules (8). However, this is unlikely since we have shown that this peptide is not effectively processed in situ and is not presented by A^k-bearing APC of the donor (5).

The molecular basis of allorecognition has been the subject of intense investigation, especially the characterization of the target structures recognized by cloned T lymphocytes. In several instances, $CD4^+$ alloreactive T lymphocytes play a crucial role in the alloresponse and have been shown to be sufficient for graft rejection (9, 10). One set of such studies on the topology of the target of alloreactive clones used single-site mutations to modify the reactivity of particular clones (4, 11, 12). It has been observed that amino acids located at a distance in the primary sequence could influence TCR recognition and/or peptide binding only when they were mutated simultaneously, supporting a model in which alloreactive T lymphocytes recognize spatially distinct residues on the α and β chains of the intact allo-MHC molecule (4, 11, 12). Alternatively, any modification of the structure of the allo-MHC target molecule could have also affected its processing and its later presentation in the form of MHC peptides.

It has been recently reported that donor MHC class I–derived peptides, in association with class I molecules or class II molecules, can be recognized by alloreactive cytotoxic cells (13–15) or $CD4^+$ alloreactive T lymphocytes (16), respectively. Here we have shown that during in vivo alloresponses, determinants on foreign MHC molecules can be presented to T cells in the form of peptides associated with self-MHC molecules. Arnold et al. (17) recently showed that mice expressing a class I (K^k) transgene, although tolerant to allogeneic MHC peptide fragments presented in association with self-MHC class I molecules (D^b), reacted at high frequency to the membrane form of the allo-MHC molecules. These results indicate that both membrane-bound intact allo-MHC molecules and allo-MHC peptides presented in the context of a self-MHC restriction element are suitable targets in allorecognition.

How crucial is donor MHC peptide presentation by recipient MHC molecules in the actual rejection process, as compared with T cell responses directed against the intact donor MHC molecules? It is well established that after transplantation, the passenger leukocytes of the donor (macrophages, Langerhans, and dendritic cells) leave the graft and can be found in the draining lymph nodes and spleen of the recipient (18). These bone marrow–derived, donor class II–positive cells then prime recipient T cells that recognize the intact allogeneic MHC molecules present on their surface. After recognition of the native allo-antigen, primed T cells of the recipient can infiltrate the graft and secrete lymphokines. For example, IFN-γ induces further class II expression on epithelial cells of the graft (19). After the passenger leukocytes have left the graft, these epithelial cells should be the only allogeneic Ia-expressing cells in the transplant. Interestingly, such non–bone marrow–derived class II–bearing cells have been demonstrated to imprint nonresponsiveness on sensitized T cell clones rather than triggering alloreactive T cell responses (20, 21). We surmise that recognition of native allo-MHC molecules is only critical for initiation of the rejection but it cannot be the mechanism for maintaining the rejection. Instead, host-derived macrophages and other class II–positive inflammatory cells infiltrate the graft (18), process donor MHC molecules, and present these allo-MHC peptides in the context of self-MHC class II. This mechanism should eventuate in completing the rejection by providing help for the production of donor-directed antibodies.

The results presented in this article demonstrate that donor class II–derived peptides can be presented by recipient MHC molecules, a process that may play a critical role in the rejection of the allograft. With respect to the therapy, while at an early stage in the rejection process, antigen presentation by donor MHC class II molecules might be an effective target; at a later point, blocking of recipient MHC class II molecules with antibodies or peptides may be an important factor in preventing the rejection.

We thank Dr. Paul. V. Lehmann for helpful discussions and Drs. Yoko Mullen and Takashi Yamagushi for technical assistance in performing the skin grafts.

This work was supported in part by National Institutes of Health grants CA-24442; AI-11183 (E. E. Sercarz), and GM-36804 (M. McMillan).

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Received for publication 28 June 1991 and in revised form 7 October 1991.

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