**Brief Definitive Report**

**Influence of T Cell Receptor Vα Expression on Mlsa Superantigen-specific T Cell Responses**

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**Summary**

Recognition of conventional foreign antigen by T cells is determined by the expression of multiple variable regions of both α and β chains of the T cell receptor (TCR) α/β heterodimer. In contrast, there exists a class of antigens that appears to interact with the TCR α/β heterodimer through the variable region on the β chain (Vβ), independent of other TCR components, a property that has led to their designation as superantigens. The goal of the present study was to analyze Vα use in Vβ6+ T cells responsive to the superantigen, Mlsa. Results indicate that while deletion of T cells expressing Vβ6 in Mlsa-expressing mice is essentially complete and therefore appears to occur regardless of Vα use, in vitro Mlsa stimulation of T cells from Mlsa-negative mice results in significant skewing of Vα use among responding Vβ6+ T cells. This indicates that Vα expression influences recognition of the superantigen, Mlsa, by mature peripheral T cells.

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T cell recognition of conventional antigens is influenced by multiple variable regions of the α/β TCR (1). There exists, however, a class of antigens that appears to interact with the TCR α/β heterodimer through the V region on the β chain (Vβ), regardless of other variable regions (V, D, J, or N regions) expressed on either the α or β chain. The ability of these antigens to elicit vigorous primary proliferative responses from T cells based solely on Vβ use has led to their designation as superantigens (2). These superantigens occur endogenously in the mouse (3), and as bacterial, mycoplasma, and viral products that act as exogenous superantigens to murine and human T cells (4-6). TCR residues implicated in superantigen recognition have been mapped to regions of the TCR β chain distant from residues that appear to be critical for conventional antigen recognition (7-9).

Endogenously expressed superantigens in the mouse, now believed to be products of mouse mammary tumor viruses (10), have extensive effects on formation of the T cell repertoire. In the majority of instances, virtually all T cells expressing a superantigen-specific Vβ are deleted in mice which express that superantigen. In other instances, e.g., Vβ11 expression, only partial deletion occurs in some strains of mice (11). A potential explanation for this nonsusceptibility to negative selection is that variable regions of the TCR other than Vβ are involved in superantigen recognition.

The present study analyzed Vα use in Vβ6+ T cells responsive to the superantigen Mlsa. Results indicate that whereas deletion of T cells expressing Vβ6 in Mlsa-expressing mice is essentially complete, regardless of Vα use, in vitro stimulation of T cells with Mlsa results in significant skewing of Vα use among responding Vβ6+ T cells, indicating that Vα expression influences recognition of Mlsa.

**Materials and Methods**

**Animals.** BALB/c Mlsa mice were generated by Berumen et al. (12). Other mice were obtained from the Frederick Cancer Research Facility (Frederick, MD).

**Reagents.** Anti-Vα2 (B20.1) (13), anti-Vα3.2 (RR3-16) (14), anti-Vβ8 (KT50) (15), and anti-Vα11 (RR8-1) (16) antibodies were as previously reported. Purified and directly conjugated anti-Vβ6 were purchased from Pharmingen (La Jolla, CA). Biotin- and FITC-conjugated anti-Thy1.2 were obtained from Becton Dickinson & Co. (San Jose, CA). FITC-conjugated goat anti-rat Ig was purchased from Caltag, (San Francisco, CA).

**Flow Cytometry Analysis.** T cells were analyzed as previously described (17).

**T Cell Stimulation.** Splenocytes were enriched for T cells by panning on rabbit anti–mouse Ig coated plates (17). 3 x 105 T cells were cultured for 48 h with 6 x 105 mitomycin C-treated spleen cells of the indicated strain, or in wells that had been coated with 10 μg/ml anti-Vβ6 mAb. After 48 h, RIL2 (Cetus Corp., Emeryville, CA) (final concentration 50 U/ml) was added directly to the cultures. Cultures were harvested 6–7 d after initiation.

**Results**

T cells expressing Vβ6 are clonally deleted during development in an environment in which Mlsa is expressed as a
self-antigen (18). We attempted to analyze the influence of TCR components other than \( \beta \) in such instances of \( \beta \)-specific negative selection by analyzing \( \alpha \) use in the few undeleted \( \beta_6^+ \) T cells remaining in AKR/J or BALB/c Mls\(^a\) (Mls\(^a\) strains) mice. However, the deletion of \( \beta_6^+ \) T cells in Mls\(^a\) mice was so complete that it was impossible to reliably analyze \( \alpha \) expression in the remaining \( \beta_6^+ \) T cells (Fig. 1).

To determine whether the response of mature T cells to endogenous superantigens such as Mls\(^a\) is determined solely by \( \beta \) expression, or whether \( \alpha \) use also plays a role, primary anti-Mls\(^a\) T cell cultures were analyzed by flow cytometry for simultaneous \( \alpha \) and \( \beta \) expression. Activation of heterogeneous T cells with Mls\(^a\)+ stimulators consistently resulted in preferential expansion of \( \beta_6^+ \) T cells (Fig. 2 A). Analysis of \( \alpha \) expression in C3H \( \beta_6^+ \) T cells demonstrated that there was a significant and consistent decrease in expression of \( \alpha_2 \) and \( \alpha_8 \) on the \( \beta_6^+ \) T cells that were expanded in anti-Mls\(^a\) primary cultures and stimulated with either AKR/J or CBA/J cells, as compared with expression in normal \( \beta_6^+ \) spleen cells (Fig. 3). \( \beta_6^+ \) T cells generated in BALB/c anti-BALB/c Mls\(^a\) cultures, in which responder and stimulator cells differed only by Mls\(^a\)-linked genes, showed similar decreases in \( \alpha_2 \) and \( \alpha_8 \) (Fig. 3).

Figure 1. Expression of Mls\(^a\) results in essentially complete deletion of \( \beta_6^+ \) T cells. Lymph node cells were stained with anti-\( \beta_2 \) or anti-\( \beta_6 \) culture supernatant and goat anti-rat-FITC followed by biotin-labeled anti-Thy 1, and Texas Red-conjugated avidin. Background staining with an irrelevant antibody has been subtracted from the percentages shown.

Figure 2. Selective expansion of \( \beta_6^+ \) T cells and analysis of \( \alpha \) expression by \( \beta_6^+ \) T cells in anti-Mls cultures. (A) BALB/c T cells from 7-d cultures stimulated with either Mls\(^a\)+ (BALB/c Mls\(^a\)) or allogeneic-MHC (B10.A) stimulator cells were analyzed for expression of \( \beta_6 \). Background staining with an irrelevant antibody has been subtracted from the percentages shown. (B) These cultures were analyzed for \( \alpha \) expression by incubation with anti-\( \alpha \) culture supernatant and goat anti-rat biotin followed by FITC-labeled anti-Thy 1 or anti-\( \beta_2 \) and Texas Red-conjugated avidin. The two-parameter profiles were then software-gated to determine \( \alpha \) expression (red fluorescence) on \( \beta_6^+ \) cells. (Dotted lines) control staining. (Solid lines) indicate staining with the appropriate anti-\( \alpha \) mAb.
To determine whether the observed changes in V\alpha expression were unique to Mls-specific responses, BALB/c responder cells were cultured with either Mls-congenic stimulators or with MHC-disparate, Mls-negative B10.A stimulators (Fig. 2 B, and Fig. 3). V\alpha expression in V\beta6+ T cells isolated from anti-B10.A cultures was not significantly different from V\alpha expression in V\beta6+ T cells from naive spleen cells, in contrast to the reduced V\alpha2 and V\alpha8 expression in anti-Mls cultures (Fig. 3).

Of the four available V\alpha-specific antibodies, only two are reactive with BALB/c and C3H T cells because of the allelic specificity of these reagents. V\alpha expression was therefore examined in Mls-reactive B10.A T cells, a strain in which T cells are reactive with all four V\alpha-specific mAbs (Fig. 4). Expression of V\alpha2, V\alpha3, and V\alpha8 in V\beta6+ cells, expressed in Fig. 4 relative to that observed in uncultured V\beta6+ spleen cells, was reduced in Mls-stimulated V\beta6+ B10.A T cells, whereas there was an increase in V\alpha11 expression. In contrast, when V\beta6+ T cells were selectively expanded by activation with an anti-V\beta6 antibody, V\alpha expression of the resulting V\beta6+ T cells was equivalent to that seen in uncultured spleen, arguing that the difference in V\alpha expression noted between Mls-activated V\beta6+ T cells and unstimulated V\beta6+ T cells was not due to a generalized decrease in the responsiveness of V\alpha2, V\alpha8, and V\alpha3-expressing T cells. Analysis of V\alpha expression in CD4+ and CD8+ T cells indicated that the differences observed could not be accounted for by selective expansion of T cell subsets in response to Mls stimulation (data not shown). These results indicate that V\beta6+ T cells expressing certain V\alpha's are selectively more or less responsive to the Mls superantigen.

Discussion

It has previously been thought, based on V\beta-specific deletion of T cells by superantigens (11), that superantigen recognition by T cells is dependent solely on the TCR V\beta gene product expressed on the T cell. However, analysis in this study of V\alpha expression in Mls-responsive T cells demonstrates that skewing of V\alpha use occurs. Although expression of a particular V\beta may be sufficient for clonal deletion, it appears that V\alpha expression plays a significant role in the ability of T cells to respond functionally to superantigens. These results are consistent with the previous studies of Pullen et al. (8) and Yui et al. (19), which suggested that TCR variable regions other than V\beta might play a role in determining responsiveness to Mls.

Expression of TCR elements other than V\alpha and V\beta was not analyzed in this study, and it remains possible that dominant influences of J, D, or N products on the response to Mls may exist. TCR variable regions other than V\beta could influence T cell recognition of the Mls superantigen either directly or indirectly. Superantigen may interact with a recognition site consisting of both \alpha and \beta chain variable regions such that inappropriate expression of one or the other would result in lack of binding and activation by the superantigen. Studies evaluating the binding site of superantigens on the TCR (7-9) have suggested that V\beta residues critical for superantigen binding are distant from the \alpha chain. However, the actual binding site has not yet been directly demonstrated. Alternatively, it is possible that superantigen does not directly contact variable regions of the TCR other than V\beta, but that expression of other regions conformationally affects the TCR and consequently affects the ability of superantigen to bind to the TCR V\beta.

Although alloreactive Mls-specific V\beta6+ T cells showed skewed V\alpha use in the present study, deletion of V\beta6+ T
cells in Mls$^+$ mice appears to affect essentially all V$\beta$6$^+$ T cells, regardless of the V$\alpha$ expressed. The in vitro differential expansion of T cells expressing specific V$\alpha$ products reflects the outcome of a competitive process in which those cells that survive and/or proliferate most efficiently predominate at the end of culture. In contrast, clonal deletion of V$\beta$6$^+$ cells during in vivo development may not reflect any such competitive process. Thus, it is possible that essentially all V$\beta$6$^+$ T cells have sufficient affinity to mediate negative selection in vivo, despite the fact that V$\alpha$ expression affects T cell recognition of Mls$^+$ in a manner that is reflected in differential in vitro responsiveness. The TCR requirements for superantigen-mediated clonal deletion may differ either quantitatively or qualitatively from the requirements for activation of mature Mls$^+$-specific T cells.

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