

Brief communications

T-cell receptor peptides that inhibit the T-cell response to allergen induce transforming growth factor- β_1 production

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Recent findings indicate restricted usage of T-cell receptor (TcR) β chain variable region 3 (V β 3) gene elements in the human polyclonal T-cell response to the allergens of house dust mite (HDM).^{1,2} Furthermore, we have reported that a peptide analog of TcR-V β 3 CDR2 specifically inhibits the polyclonal proliferative response of human V β 3+T cells to unfractionated *Dermaphagoides pteronyssinus* extract.² Although the mechanism of inhibition remains to be defined, there is increasing evidence that immunosuppressive cytokines may play an important role in the downregulation of immune responsiveness in a number of experimental systems.³⁻⁵ In this study we reveal that the V β 3-CDR2 peptide induces the production of the inhibitory cytokine transforming growth factor- β_1 (TGF- β_1) but that no IL-4, IL-10, or interferon- γ (IFN- γ) activity was detectable in the same culture supernatants.

METHODS

Separation of peripheral blood mononuclear cells (PBMCs) from healthy adults by Ficoll-Hypaque density centrifugation and CD4⁺ and CD8⁺ T-cell depletion protocols (routinely <3% contamination) has recently been described.² PBMCs (5×10^6) were cultured in 1 ml of supplemented RPMI-1640 media² (Gibco, Paisley, Scotland) and 5% AB⁺ human serum with 10 μ g/ml

Abbreviations used

HDM:	House dust mite
IFN- γ :	Interferon- γ
MTSE:	<i>Mycobacterium tuberculosis</i> soluble extract
PBMCs:	Peripheral blood mononuclear cells
TcR:	T-cell receptor
TGF- β_1 :	Transforming growth factor- β_1
V β :	Variable region of the β chain of the T-cell receptor

Mycobacterium tuberculosis soluble extract (MTSE),² 25 μ g/ml HDM extract,² 25 to 100 μ g/ml V β 3-CDR2 peptide (GLGLRLIYFSYDVKMKEKEDI), V β 6.7a-CDR2 peptide (SPLGSKDPASNG), murine V β 8-CDR2 peptide (IDGKETSDAVYSYHIL),² or 10 μ g/ml phytohemagglutinin plus 10 ng/ml phorbol myristate (PHA/PMA; Sigma Chemical Co., St. Louis, Mo.). Supernatants from these cultures were harvested after 5 days and frozen at -20°C until they were tested for cytokine activity as described below.

IL-4 and IFN- γ activity were measured with ELISAs (Eurogenetics, Teddington, U.K.). IL-10 was measured by ELISA with antibodies kindly provided by Dr. Anne O'Garra (DNAX, Palo Alto, Calif.). The Mv1Lu bioassay was used to measure TGF- β_1 activity.⁶ Latent TGF- β_1 was converted to the active form by reducing the pH to less than 3.0 for 30 minutes at room temperature. TGF- β_1 activity was inversely proportional to Mv1Lu cell growth, as determined by tritiated thymidine incorporation,² when tests were performed in replicates of 3 to 6 wells, with standard deviations routinely less than 20%.

RESULTS

Supernatants harvested from PBMCs cultured with 25 to 100 μ g/ml V β 3-CDR2 peptide were tested in the Mv1Lu growth inhibition assay for TGF- β_1 bioactivity. The V β 3-CDR2 peptide induced a dose-dependent production of active

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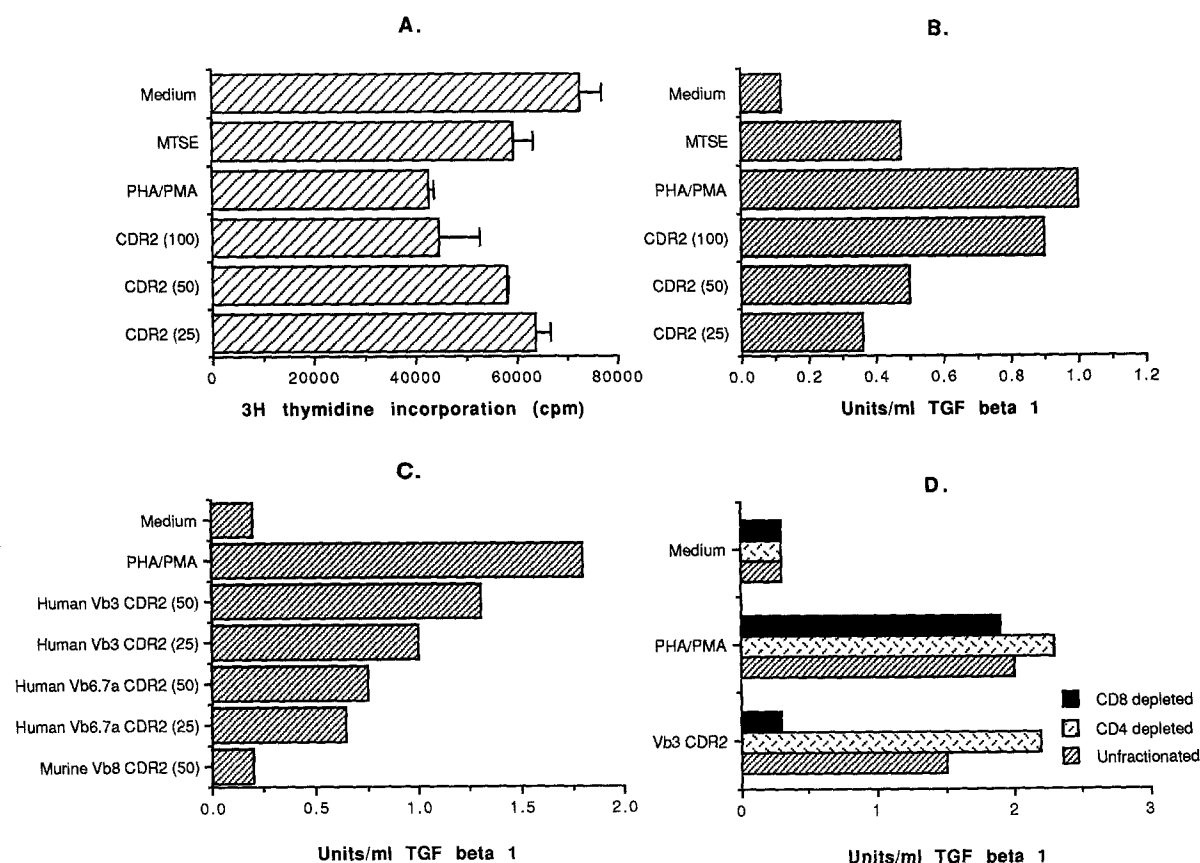


FIG. 1. Induction of TGF- β_1 activity by TcR peptides. Unfractionated human PBMCs (A-D) or CD4- and CD8-depleted PBMCs (D) were cultured at 5×10^6 cells/ml in medium, with 10 μ g/ml PHA plus 10 ng/ml PMA, 10 μ g/ml MTSE, or the indicated concentrations (25 to 100 μ g/ml; 50 μ g/ml in D) of TcR peptides for 5 days. Culture supernatants were then harvested and assayed for TGF- β_1 bioactivity as described in Methods. Data are presented in A as mean counts per minute $\pm$ standard deviation of replicate cultures and B, C, and D as units per milliliter of TGF- β_1 activity as extrapolated from a standard curve obtained with recombinant human TGF- β_1 . A and B represent data from the same experiment.

TGF- β_1 , with levels comparable to those induced by PHA plus PMA, a potent stimulus of TGF- β_1 production, but more than those induced by antigen (Fig. 1, A and B). Comparable results were observed in all nine individuals tested (mean $\pm$ SD): TGF- β_1 in medium, 0.31 ± 0.24 U/ml; PHA/PMA, 1.52 ± 0.85 U/ml; TcR-V β_3 CDR2 peptide, 1.43 ± 0.79 U/ml; and HDM, 0.585 ± 0.53 U/ml. A second CDR2 peptide derived from the human V $\beta_6.7a$ gene element induced lower but detectable levels of TGF- β_1 in two individuals tested (Fig. 1, C, and data not shown). This was particularly interesting because V $\beta_6.7a$ -TcR usage was detected, albeit at a lower level than TcR-V β_3 , in a panel of HDM-specific T-cell clones.¹ A control murine CDR2 peptide, derived from murine TcR-V β_8 , failed to stimulate TGF- β_1 production (Fig. 1, C).

To demonstrate that the TGF- β_1 bioactivity detected in our cultures was directly synthesized after peptide stimulation of PBMCs and not caused by serum contaminants, cells were cultured for 24 hours with various stimuli in serum containing medium, washed extensively, and then recultured in the absence of serum for an additional 3 days. Serum-free supernatants were harvested and assayed for active versus total (latent plus active) TGF- β_1 content after in vitro conversion of latent TGF- β_1 by acid treatment. Supernatants from PBMCs cultured in medium alone or with V β_3 -CDR2 peptide contained less than 0.3 and 2.4 active total U/ml TGF- β_1 compared with 1.4 and 13 U/ml TGF- β_1 , respectively.

Cell depletion studies previously suggested that CD8⁺ T cells play a central role in mediating CDR2 peptide inhibition of the T-cell response to

HDM allergens.² The induction of active TGF- β_1 in unfractionated, CD4-depleted, and CD8-depleted PBMCs was therefore compared. As shown in Fig. 1, D, a representative experiment of four performed, high levels of TGF- β_1 bioactivity were detectable in all three groups after PHA/PMA stimulation. In contrast, V β 3-CDR2 peptide induced TGF- β_1 production in unfractionated and CD4-depleted, but not in CD8-depleted, PBMC cultures, suggesting that the CD8⁺ T-cell fraction is the major cell type responding to this TcR peptide. When IL-10 levels were measured in the same supernatants, only PHA/PMA stimulation resulted in marked cytokine production, as measured by ELISA. Furthermore, induction of IL-10 by PHA/PMA was readily detected in unfractionated and CD8-depleted (6.7 and 4.1 U/ml respectively), but not CD4-depleted cultures (0.6 U/ml; medium control, HDM, or CDR2 peptide for all groups <1.0 U/ml).

The V β 3-CDR2 peptide preparation also failed to induce IFN- γ ($n = 6$; medium, 3.8 ± 4.5 ; PHA/PMA, 329 ± 47.8 ; CDR2, 2.8 ± 2.8 ; HDM, 8.2 ± 9.5 ; MTSE [$n = 4$], 247.5 ± 129 pg/ml) or IL-4 (medium, HDM, or CDR2 peptide <6 pg/ml; PHA/PMA, 40 pg/ml) production as measured by specific ELISA. IL-2 activity, measured in the CTLL growth assay, was also not detected in 24-hour culture supernatants of cells cultured in medium or peptide, but was found in the control HDM and PHA/PMA supernatants (data not shown).

DISCUSSION

In this report we demonstrate that peptides derived from the human TcR-V β 3 CDR2 region induce production of active TGF- β_1 by PBMCs in culture. IL-10, IFN- γ , IL-4, or IL-2 activity in the same cultures was below the limits of detection. Strong induction of all cytokines was observed after stimulation with PHA/PMA. Furthermore, depletion of CD8⁺ T cells resulted in loss of TGF- β_1 production, suggesting that CD8⁺ T cells were responsible for TGF- β_1 production after TcR peptide stimulation. Similarly, inhibition of the proliferative response of human PBMCs to HDM allergens by V β 3-CDR2 peptide required the presence of CD8⁺ T cells. Our findings suggest that this inhibition may, in part, be mediated through the induction of immunosuppressive cytokines, such as TGF- β_1 . Nevertheless, neu-

tralizing anti-TGF- β_1 antibody failed to reverse CDR2 peptide-mediated inhibition of the HDM response. This observation is not surprising, given the likely requirement for close interaction of two different cell types, in this case the putative CD8⁺ regulatory T cells with CD4⁺, TcR-V β 3⁺ HDM-reactive cells, for the conversion of latent to active TGF- β_1 activity as proposed in other experimental systems.⁷ If this is so, it is unlikely that "free" cytokine would be available for antibody neutralization.

TGF- β_1 acts directly on T cells by inhibiting IL-1 and IL-2 receptor expression and cytokine production, as well as indirectly by inhibiting T-cell activation by modulation of antigen-presenting cell function.³ In addition, TGF- β_1 inhibits the switch to germline ϵ transcription and IgE production, while selectively inducing a switch to α chain and IgA transcription. A number of inhibitory mediators induce production of TGF- β_1 , including cyclosporine,⁵ the synthetic glucocorticoid dexamethasone,⁴ and, most recently in this report, TcR peptides. These observations have led to growing recognition of an important role for this immunosuppressive cytokine in the modulation of allergic and other immune phenomena.

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