Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 11342–11346, December 1992
Immunology

Thymic depletion and peripheral activation of class I major histocompatibility complex-restricted T cells by soluble peptide in T-cell receptor transgenic mice

CLIO MAMALAKI*, TRISHA NORTON*, YUJIRO TANAKA*, ALAIN R. TOWNSEND†, PHILLIP CHANDLER‡, ELIZABETH SIMPSON‡, AND DIMITRIS KIOUSSIS*

*Laboratory of Molecular Immunology, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA, United Kingdom; †Institute of Molecular Medicine, John Radcliffe Hospital, Headington, Oxford OX3 9DU, United Kingdom; and ‡Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, United Kingdom

Communicated by Avrion Mitchison, August 14, 1992

ABSTRACT **Injection of mice transgenic for a class I major histocompatibility complex-restricted T-cell receptor with a soluble peptide antigen from influenza virus nucleoprotein results in clonal depletion of double-positive immature thymocytes in the thymus and activation of mature T cells in the periphery, accompanied by a transient up-regulation of the T-cell receptor and CD3 and CD8 coreceptor molecules.**

During differentiation in the thymus, thymocytes are subject to positive or negative selection resulting in both self-major histocompatibility complex (MHC) restriction and self-tolerance (1–4). In the periphery, exposure of mature T cells to antigen recognized by their receptor (the T-cell receptor, TCR) normally leads to activation (5). Class I MHC molecules present to the TCR primarily peptides from proteins that have been synthesized within the cell (6). In contrast, class II MHC molecules present peptides from proteins endocytosed by the presenting cell (7, 8). During development thymocytes may be required to become tolerant to self-proteins apparently not synthesized in the thymus (9). While it is possible that peptides from the periphery reach the thymus, where they can be presented by class II MHC molecules, it is not clear whether the class I-restricted T-cell repertoire could also be affected by such a mechanism under physiological or experimental conditions.

In recent years studies using transgenic mice that express a single antigen-specific receptor on most of their T cells have shed considerable light on the mechanisms by which positive and negative selection processes occur (10–13). Murphy and his colleagues (14) generated transgenic mice that express α TCR recognizing a chicken ovalbumin peptide (OVA323–339) associated with the MHC class II molecule I-A^d and injected them with OVA323–339. In adult TCR transgenic mice this resulted in rapid deletion of $CD4^+CD8^+$ (abbreviated $CD4^+8^+$) TCR^{lo} thymocytes, and apoptosis was observed within 20 hr of treatment (14). This result was in accordance with the generally accepted mode of exogenous antigen presentation by class II MHC molecules. Recently we developed a transgenic mouse that expresses the TCR from a cytotoxic T-cell clone (F5) recognizing in the context of D^b a peptide of the influenza virus A/NT/60/68 nucleoprotein [NP-(366–379)] (15, 16). The F5 receptor utilizes the $V_\alpha 4$ and $V_\beta 11$ members of the α and β chain variable-region gene families (17). The majority of $CD8^+$ T cells from these animals express the transgenic TCR and recognize antigen in association with the appropriate MHC molecule (C.M., unpublished results). F5 TCR transgenic mice thus represent an ideal model in which to determine whether exogenously administered peptide could affect the development of thymocytes restricted by class I MHC. Thus, we injected the transgenic mice with a synthetic oligopeptide consisting of amino acids 366–374 of the influenza nucleoprotein, which can trigger activation of specific T cells and sensitize targets for cytotoxicity assays (16).

MATERIALS AND METHODS

Generation of F5 TCR Transgenic Mice. The cDNAs for the F5 α and β chains (17) were inserted in an artificial *Eco*RI site at the 5′ untranslated region of a human CD2 minigene (18) that has a frameshift mutation in the CD2 coding region (C.M., unpublished data). The chimeric α and β genes were coinjected into C57BL/10 (abbreviated B10) mouse embryos as described (19).

Peptide Treatment of F5 TCR Transgenic Mice. Mice 6 weeks old were injected intraperitoneally with phosphate-buffered saline (PBS) or peptide solutions in the same medium. The following peptides were used: the 9-mer Ala-Ser-Asn-Glu-Asn-Met-Asp-Ala-Met [NP-(366–374)], the 14-mer Ala-Ser-Asn-Glu-Asn-Met-Asp-Ala-Met-Glu-Ser-Ser-Thr-Leu [NP-(366–379)] (both from nucleoprotein of influenza virus A/NT/60/68), and the 9-mer Ala-Ser-Asn-Glu-Asn-Met-Glu-Thr-Met [NP-(366–374)] (from nucleoprotein of influenza virus A/PR/8/34). The oligopeptides were synthesized on an Applied Biosystems 430A peptide synthesizer.

Flow Cytometry. For two-color flow cytometry, 10^6 thymocytes or lymph node cells were stained with phycoerythrin-conjugated anti-CD4 monoclonal antibody GK1.5 (Becton Dickinson) and fluorescein isothiocyanate-conjugated anti-CD8 monoclonal antibody 53-6.7 (Becton Dickinson).

For three-color analysis, 10^6 thymocytes or lymph node cells were stained with fluorescein isothiocyanate-conjugated anti-CD8 (53-6.7; Becton Dickinson), anti-CD4-RED613 (YTS 191.1.2; GIBCO/BRL), and biotinylated KTII anti-$V_\beta 11$ (gift from Kyuhei Tomonari, Clinical Research Centre) (20), followed by phycoerythrin-conjugated streptavidin (Bio Genesis, Bournemouth, U.K.).

Alternatively, cells were stained with phycoerythrin-conjugated anti-CD8 (Ly2-RD1; Coulter), anti-CD4-RED613 (YTS 191.1.2; GIBCO/BRL), and fluorescein isothiocyanate-conjugated anti-CD3 (2CII) (gift from R. Zamoyska) antibodies. Three-color FACS analysis was performed with a FACS-scan laser instrument and Lysis I program (Becton Dickinson).

Mixed Lymphocyte Reactions (MLRs) and Cytotoxicity Assay. Responder suspensions of spleen cells in RPMI 1640 medium supplemented with 10% fetal calf serum, 50 μM

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Abbreviations: MHC, major histocompatibility complex; TCR, T-cell receptor; MLR, mixed lymphocyte reaction; CML, cell-mediated lysis; IL-2, interleukin 2.

2-mercaptoethanol, penicillin at 100 international units/ml, streptomycin at 100 μg/ml, 10 mM Hepes at pH 7.2, and 2 mM glutamine were dispensed at 1×10^6 cells per 4-ml well for cell-mediated lysis (CML) cultures and 1×10^4 cells per 0.2-ml flat-bottomed microtiter well for proliferative MLR cultures. Recombinant human interleukin 2 (IL-2) was added to a final concentration of 50 international units/ml for CML cultures and 10 international units/ml for MLR cultures. Cells used as antigen were spleen cells from which erythrocytes had been removed by brief exposure to hypotonic shock. B10 spleen cells were used either alone (B10) or after 45 min of incubation with 100 μM peptide [NP-(365–379)] followed by two washes in RPMI 1640 medium ($B10^P$). These were irradiated with 2500 roentgens (1 roentgen = 0.26 mC/kg) from a ^{60}Co source; 1×10^6 antigen cells were added to each 4-ml CML culture well, 1×10^5 antigen cells to each 0.2-ml microtiter well for the [^{3}H]thymidine MLR assay. MLR plates pulsed at 72 hr with [^{3}H]thymidine at 1 μCi (37 kBq) per well were harvested 6 hr later for β scintillation counting. CML cultures (attacker cells) were harvested after 5 days, and cells were counted and adjusted to a concentration of 3×10^6/ml, then dispensed in triplicate into round-bottomed microtiter wells. They were then serially diluted 1:3 in the microtiter plate to give four concentrations, each in triplicate. Then 1×10^4 ^{51}Cr-labeled EL-4 ($H\text{-}2^b$) target cells were added to each well and the plates were centrifuged at 50 × *g*. Each attacker cell suspension was assayed on EL-4 targets alone (EL-4) and EL-4 targets sensitized by preincubation with 50 μM NP-(365–379) ($EL\text{-}4^P$), at attacker-to-target (A:T) ratios of 30:1, 10:1, 3:1, and 1:1. After a 3-hr incubation period supernatants from the CML assay was harvested for γ counting. Percent specific lysis was computed according to the formula % specific lysis = [(Exp − C)/(Max − C)] × 100, where Max = cpm from target cells incubated in 5% Triton X-100, C = cpm from target cells incubated in medium, and Exp = cpm from target cells incubated with attacker cells. Twelve-point regression analysis was performed and the value for percent specific lysis at 10:1 was taken. Significant lysis was over 10%, from a titrating regression curve with a coefficient of variation, r^2, between 0.80 and 1.00.

RESULTS

Soluble Peptide Causes Depletion of Thymocytes and Expansion and Activation of Peripheral T Cells. To study the effects of cognate peptide on the differentiation of thymocytes and on peripheral T cells, 6-week-old F5 TCR transgenic mice were injected intraperitoneally with PBS or 50 nmol of peptide daily for 4 days. Thymocytes and lymph node T cells were isolated and stained with monoclonal antibodies for CD4, CD8, CD3, and $V_\beta 11$ and analyzed by flow cytometry. Fig. 1 shows the results from such an experiment. PBS-injected mice showed the same T-cell subpopulation distribution as untreated transgenic littermates, with a predominance of single-positive $CD8^+$ cells over single-positive $CD4^+$ cells in the thymus (Fig. 1*A*) and lymph nodes (Fig. 1*B*), reflecting positive selection occurring in these mice. Mice injected with peptide, however, showed a marked decrease of cell number in the thymus (Fig. 2*A*). The absolute number of all subpopulations was decreased, the $CD4^+8^+$ subpopulation being affected the most (Fig. 2*B*). Of the remaining cells in the thymus the majority (70%) had a $CD4^-8^+$ phenotype (Fig. 1*A*).

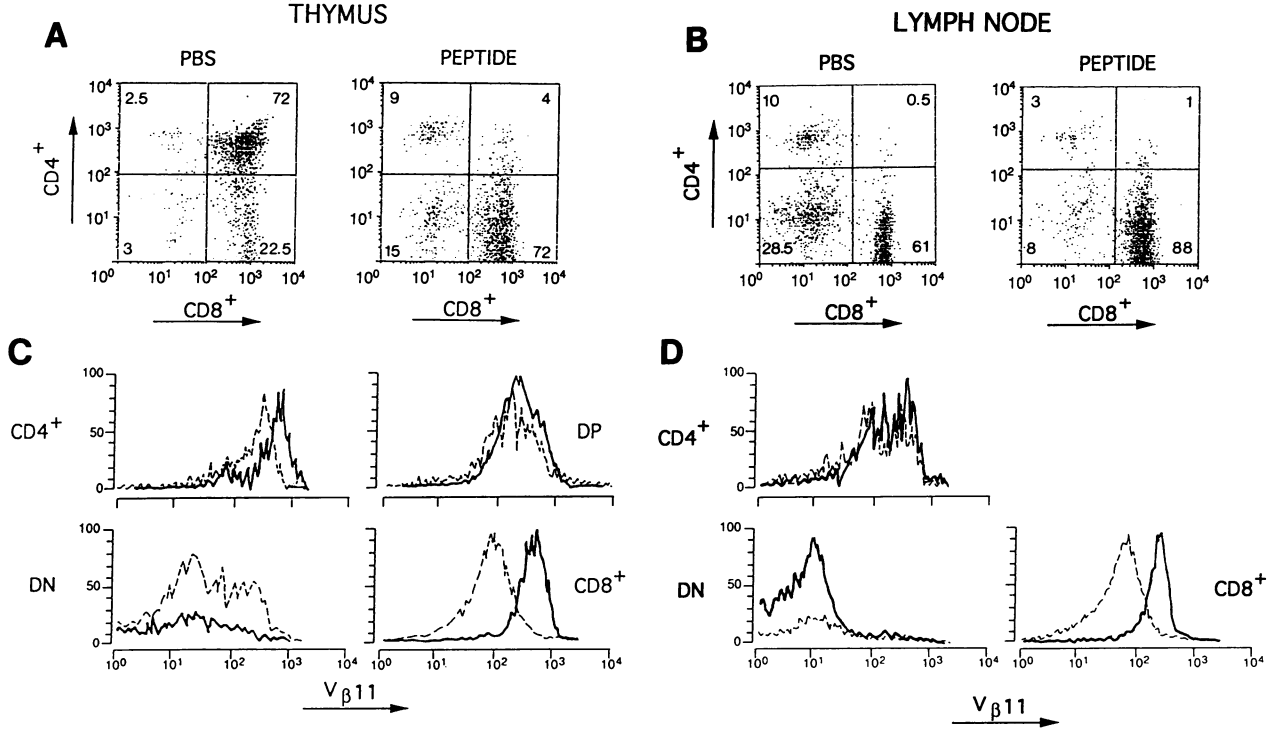

FIG. 1. (*A*) Preferential depletion of immature $CD4^+8^+$ thymocytes from the thymus of F5 TCR transgenic mice injected with antigen. Six-week-old transgenic mice expressing the F5 TCR were injected daily for 4 days with PBS or 50 nmol of a synthetic oligopeptide (residues 366–374) from the nucleoprotein of influenza virus A/NT/60/68 in PBS. The thymocytes (10^6) were stained with anti-CD8, anti-CD4-RED613, and KTII anti-$V_\beta 11$ antibodies followed by phycoerythrin-conjugated streptavidin. Numbers in the quadrants are percent of the total sample. (*B*) Lymph node cells from mice described for *A* and stained with the same reagents. (*C*) Expression of $V_\beta 11$ on the different subpopulations in the thymus of mice injected with PBS (solid line) or 50 nmol of peptide (broken line). Mice were treated as described for *A*. Cells were stained as described above and $CD4^-8^-$ (double-negative, DN), $CD4^+8^+$ (double-positive, DP), $CD4^+8^-$ ($CD4^+$), and $CD4^-8^+$ ($CD8^+$) cells were gated and the levels of $V_\beta 11$ expression were determined. (*D*) Expression of $V_\beta 11$ on the different subpopulations in lymph node cells from mice described and stained as for *A*.

The expression of the transgenic $V_\beta 11$ was affected differently in the various thymic subpopulations. Thus after 4 days of daily injections of the cognate peptide, the $V_\beta 11$ levels were significantly decreased on the $CD4^-8^+$ subpopulation. The levels were less affected on the $CD4^+8^-$ population, while on the double-positive $CD4^+8^+$ population the levels were the least affected (Fig. 1*C*). Among the $CD4^-8^-$ population we observed an increase in the proportion of cells expressing $V_\beta 11$. Whether these arose from more mature cells which down-regulated the CD4 and/or CD8 molecules or whether they existed before but were enriched because of the preferential depletion of other populations in the thymus is unclear at the moment.

In the periphery, the number of spleen cells increased 3-fold (data not shown), and the proportion of $CD8^+$ cells in the lymph nodes was increased from about 62% to 90% (Figs. 1*B* and 2*C*). The expression of $V_\beta 11$ on the lymph node cells was decreased on the $CD8^+$ T cells, whereas the levels of $V_\beta 11$ on the $CD4^+$ T cells appeared unaffected (Fig. 1*D*). The size of thymocytes and lymph node cells was increased considerably in these mice, as measured by forward and side scatter FACS analysis (data not shown), indicating activation.

Functional studies confirmed that activation had been triggered by *in vivo* peptide administration. Spleen T cells from untreated F5 transgenic mice can kill target cells in an antigen-dependent manner, but only after a period of culture *in vitro* in the presence of IL-2 (C.M., P.C., and E.S., unpublished data). In contrast, spleen cells from F5 transgenic mice injected daily for 4 days with 50 nmol of the nucleoprotein peptide were able to kill targets immediately after removal from the animal (day 0, Table 1, responders 4 to 10), without any period of culture *in vitro* with IL-2. We concluded from these experiments that peripheral T cells from F5 TCR transgenic mice are activated after intraperitoneal administration of the cognate peptide in soluble form.

Thymic Depletion and Peripheral Activation Are Peptide Specific and Dose Dependent. To determine the lowest dose which would cause the effects described above, F5 TCR transgenic mice were injected intraperitoneally with amounts of peptide varying from 1 nmol to 50 nmol daily for 4 days. Thymocytes and lymph node cells were analyzed as described above. As shown in Fig. 2, the absolute numbers of thymocytes are decreased in a manner dependent on the levels of injected peptide, with 1 nmol daily for 4 days apparently not causing thymocyte depletion, while doses of 10, 25, and 50 nmol produced profound effects. In a separate experiment (FACS data not shown), of two mice injected with 3.5 nmol of peptide daily for 4 days only one showed signs of thymocyte depletion. Notably, peripheral T cells from this mouse were activated and able to kill targets in an antigen-dependent manner without a period of culture *in vitro* with IL-2 (Table 1, responder 4). With doses of peptide at which there was a reduction of total thymocyte numbers, the double-positive $CD4^+8^+$ was the population most affected. In the periphery injection of peptide in amounts higher than 3.5

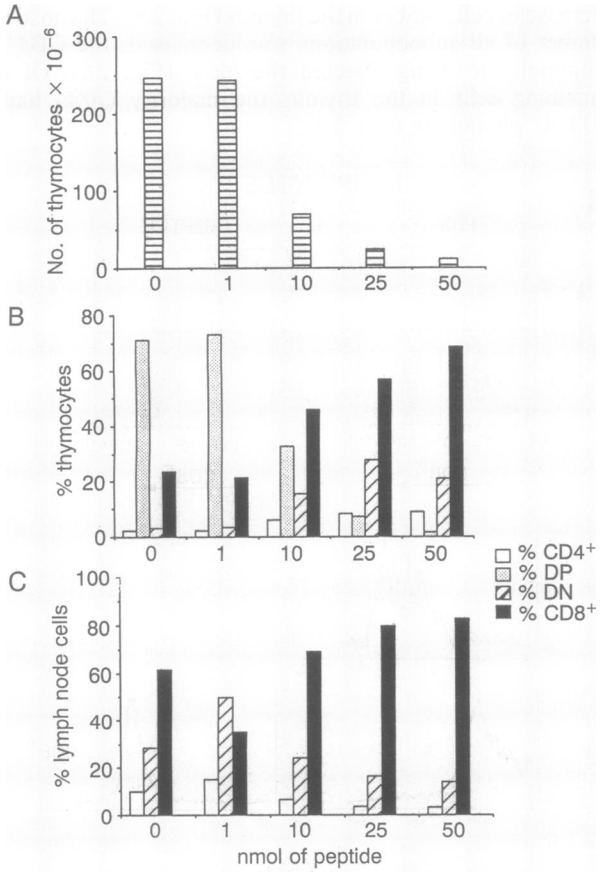

FIG. 2. Absolute numbers (*A*) and proportional analysis of the $CD4^-8^-$ (DN), $CD4^+$, $CD8^+$, and $CD4^+8^+$ (DP) subpopulations of thymocytes (*B*) or lymph node cells (*C*) in transgenic mice injected with PBS or peptide. F5 TCR transgenic mice were injected daily for 4 days with PBS or with 1, 10, 25, or 50 nmol of a synthetic oligopeptide (residues 366–374) from the nucleoprotein of influenza virus A/NT/60/68. Thymocytes and lymph node cells were stained with anti-CD4 and anti-CD8 monoclonal antibodies. The bars represent the average of two mice per point in one experiment. Similar results were obtained in repeated experiments.

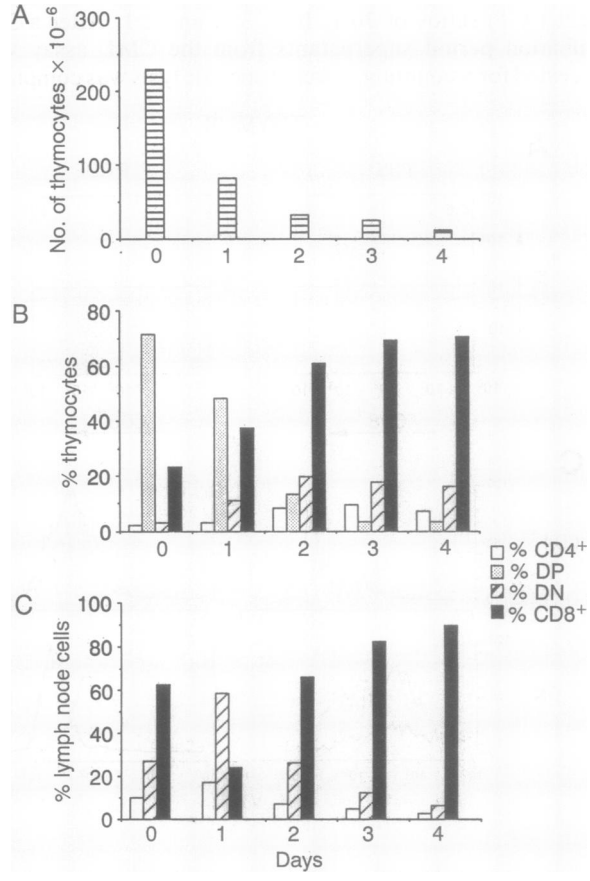

FIG. 3. Absolute numbers (*A*) and proportional analysis of the $CD4^-8^-$ (DN), $CD4^+$, $CD8^+$, and $CD4^+8^+$ (DP) subpopulations of thymocytes (*B*) or lymph node cells (*C*) in transgenic mice injected with PBS or peptide. Five- to six-week-old F5 TCR transgenic mice were injected daily for 1, 2, 3, or 4 days with 50 nmol of peptide (residues 366–374) of the nucleoprotein from influenza virus A/NT/60/68 or PBS for 4 days ("0 days"). Thymocytes and lymph node cells were stained and analyzed as described for Fig. 2.

Table 1. Peptide-specific cytotoxicity and proliferative response of spleen cells from untreated F5 transgenic mice or F5 mice treated with PBS or influenza virus nucleoprotein peptide

Responder			Cytotoxicity at day 0				Cytotoxicity at day 5		cpm of [^{3}H]thymidine incorporation after culture	
No.	Mouse	Peptide injected, nmol	EL-4	EL-4^P	Antigen *in vitro*	% recovery* at day 5	EL-4	EL-4^P	With B10	With B10^P
1	F5	None	0	3.2	B10	20	0	*27*	107	*4587*
					B10^P	107	9	*43*		
2	F5	PBS	0	6.3	B10	20	5	*37*	190	*3354*
					B10^P	52	9	*61*		
3	F5	3.5	0	3.7	B10	10	1	*48*	65	ND
					B10^P	42	8	*27*		
4	F5	3.5	0	*29.2*	B10	30	0	*49*	296	*2837*
					B10^P	35	3	*52*		
5	F5	12.5	0	*39.4*	B10	20	0	*51*	421	*3398*
					B10^P	30	0	*63*		
6	F5	12.5	0	*33.7*	B10	25	1	*42*	292	*1504*
					B10^P	25	0	*50*		
7	F5	25	0	*34.3*	B10	25	0	*54*	572	*2702*
					B10^P	30	0	*52*		
8	F5	25	0	*37.8*	B10	27	0	*48*	709	*4393*
					B10^P	25	0	*47*		
9	F5	50	0	*29.6*	B10	27	0	*48*	383	*3478*
					B10^P	27	0	*53*		
10	F5	50	0	*39.3*	B10	25	0	*49*	754	*2417*
					B10^P	30	0	*58*		
11	CBA anti-B10.A(2R)	None	*35*	*36*	B10		*58*	*51*		

Numbers in italics are significant levels of lysis or proliferation (see *Materials and Methods*). ND, not done.
*(Number of cells put in culture well ÷ number of cells recovered on day of assay) × 100.

nmol daily for 4 days resulted in an expansion of the CD8$^+$ population of T cells carrying the transgenic V_β11 chain (e.g., Fig. 1 *B* and *D*). One nanomole of peptide daily for 4 days resulted in a decrease in the proportion of CD8$^+$ in the lymph nodes. At the moment the significance of this observation is unclear. Administration of a peptide deriving from the nucleoprotein [NP-(366–374)] of influenza virus A/PR/8/34, which binds the D^b MHC molecule but is not recognized by the F5 TCR (16), had no effect (data not shown).

We conclude from these data that the minimal dose of soluble peptide administered intraperitoneally that can cause thymic depletion and peripheral T-cell activation is of the order of 3–5 nmol daily for 4 days or 0.25 nmol/g. This concentration compares with 10^{-12} M for 50% maximal killing in experiments *in vitro*. Such apparent discrepancy could be explained by differences in the experimental conditions *in vitro* and *in vivo* (21). In this model system it appears that the same concentration is required for the depletion and activation, as we have not observed one in the absence of the other. This appears to be at variance with other *in vivo* or *in vitro* systems, where negative selection appears to be more sensitive than activation (22, 23). It is likely that this difference reflects our inability to measure the effective local concentration of the peptide in spleen, where stimulation is assayed, and thymus, where depletion is observed.

Exposure to Soluble Peptide *in Vivo* Results in a Transient Increase of TCR and Coreceptor Levels on Peripheral T Cells. To investigate the kinetics of the peptide-induced effects, mice were injected intraperitoneally with 50 nmol of peptide daily for 1, 2, 3, or 4 days, and the phenotypes of thymocytes and lymph node cells were analyzed. On day 1 the total number of thymocytes in peptide-treated mice was decreased to one-third that found in PBS-treated transgenic mice (Fig. 3*A*). The decrease of the CD4$^+$8$^+$ population was detected within 1 day but became more pronounced in subsequent days (Fig. 3*B*). Although the proportion of CD4$^+$ and CD8$^+$ single-positive subpopulations increased (Fig. 3*B*), the absolute numbers of these cells were significantly reduced.

In the periphery the findings were different. The total number of splenocytes was decreased to approximately half of that found in PBS-treated mice during the first day, but increased by up to 3-fold after 4 days of daily peptide injections (data not shown). Phenotypic analysis of lymph node cells showed a decrease by one-third in the proportion of CD8$^+$ cells on day 1, then an increase during the following days to approximately 90% (Fig. 3*C*). It is not yet clear whether the decrease in numbers and proportion of the CD8$^+$ cells in the periphery during day 1 is due to death or sequestration of the reacting cells. Similar changes were observed in spleens (data not shown). We conclude from these data that the described effects are evident within 24 hr after administration of 50 nmol (2.5 nmol/g). The changes in the proportions of the different subpopulations were accompanied by remarkable changes in the density of the transgenic TCR and its coreceptor CD8. Unexpectedly, in the lymph nodes a substantial increase of the density of V_β11 and CD8 molecules was observed during day 1, followed by gradual decrease to levels below those found on PBS-treated mice by day 4 (Figs. 1*D* and 4). The size of lymph node T cells increased from day 1 of peptide administration onwards (FACS data not shown). However, the increase in CD8 and V_β11 fluorescence during day 1 was not due to the increased size of the cells, as other T-cell molecules such as CD2 and Thy-1 were not similarly affected (data not shown). In all cases the CD3 levels on thymocytes and T cells followed the pattern of V_β11 expression.

DISCUSSION

The results presented here show that injection of soluble cognate peptide into mice transgenic for a class I MHC-restricted TCR leads to thymocyte depletion, with the double-positive CD4$^+$8$^+$ population being the most dramatically affected, and to the functional activation in the periphery of cytotoxic T cells accompanied by transient up-regulation of their TCR and CD8 coreceptor.

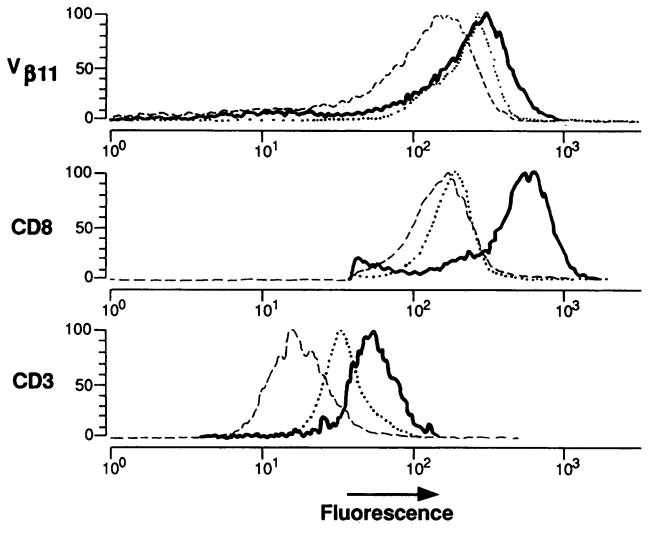

FIG. 4. Expression of $V_{\beta}11$, CD8, and CD3 molecules on lymph-node cells from F5 TCR transgenic mice injected daily with PBS for 1 or 4 days (dotted line) or with 50 nmol of nucleoprotein peptide for 1 day (heavy solid line) or 4 days (broken line). Cells were stained with anti-CD4, anti-CD8, and anti-$V_{\beta}11$ or anti-CD3 antibodies and three-color FACS analysis was performed. $CD4^-8^+$ cells were gated and the expression of $V_{\beta}11$, CD8, and CD3 was determined.

These effects were seen only with a nonameric peptide. A peptide [NP-(366–379)] 14 amino acids long which can successfully be recognized by F5 CTL clone or F5 transgenic cells when loaded on target cells (16) did not have any effect on transgenic mice when administered similarly (data not shown). This may be due to the fact that the 14-mer dissociates from D^b 100 times faster than the 9-mer (21) and is in agreement with published results indicating that the ideal length for peptides to bind onto class I MHC molecules is 8 or 9 amino acids (24). The *in vivo* effect is peptide specific: injection of F5 transgenic mice with a nonamer peptide from the homologous region of the nucleoprotein of the 1934 strain (A/PR/8/34) of influenza virus, which is not recognized by the transgenic receptor (16) although it differs from the peptide from the 1968 virus in only two amino acids, had no effect on the thymocytes or peripheral T cells of the transgenic mice (data not shown). Peptide specificity is also shown by the dose dependency (Fig. 2 and Table 1) and is consistent with the finding that nontransgenic mice treated similarly showed no changes in their T-cell subpopulations (data not shown).

These findings are consistent with the idea that in this model system relatively high levels of exogenously administered antigenic peptide can be presented by class I MHC molecules in the thymus and cause clonal depletion of thymocytes carrying the specific receptor, either by death or by rapid maturation of thymocytes and their export from the thymus. However, it is not clear whether this mechanism operates during self-tolerance induction, since the presence of specific nonamer peptides from self-proteins at high concentrations is unlikely. The findings cannot be accounted for by the massive activation of mature T cells and the subsequent release of cytokines or putative stress factors which might have a similar effect on the thymus, as no effect was observed in the thymus of nontransgenic mice that received 10^8 F5 TCR transgenic spleen cells (intraperitoneally) and were subsequently injected on the same day with NP-(366–374) peptide (data not shown).

In the periphery, treatment with peptide leads to activation of the cognate T cells, as judged by their size increase and their ability to kill target cells in the absence of a period of antigen-specific stimulation *in vitro*. The observed initial increase of TCR and CD8 levels on the cell surface may represent activation events taking place upon exposure *in vivo* to specific antigen, and this differs from the down-regulation of CD8 molecules on encounter with antigen seen in different circumstances *in vitro* (25) and *in vivo* (26). Our findings may reflect the events on the initial exposure of T cells to their antigen in the periphery. However, due to the low frequency of such precursor cells in normal mice as opposed to TCR transgenic mice this phenomenon may have eluded attention so far. The subsequent decrease in the surface density of these molecules may be attributed to the onset of a form of anergy (26–30). It is unclear how the effects on the thymus and the periphery are brought about. Possibly the peptide diffuses in a soluble form and reaches these organs, where specialized cells present it. Alternatively, presenting cells from the peritoneum may be loaded with the injected peptide and then migrate to the thymus and peripheral lymphoid tissue. Our findings support the idea that exogenously administered peptide can affect the thymic repertoire by deleting the peptide-specific $CD4^+8^+$ cells. This is probably accomplished in a manner analogous to that proposed for the removal of $CD4^+8^+$ cells that recognize self-peptides presented by MHC class II molecules (14).

We thank Drs. K. Tomonari and R. Zamoyska for directly conjugated antibodies and Ms. M. Ericsson for secretarial assistance. Y.T. was supported by the Leukaemia Research Fund.

1. von Boehmer, H. (1990) *Annu. Rev. Immunol.* **8**, 531–556.
2. Loh, D. Y. (1991) *New Biol.* **3**, 924–932.
3. Blackman, M., Kappler, J. & Marrack, P. (1990) *Science* **248**, 1335–1341.
4. Sprent, J., Gao, E.-K. & Webb, S. R. (1990) *Science* **248**, 1357–1363.
5. Zinkernagel, R. M. & Doherty, P. C. (1979) *Adv. Immunol.* **27**, 51–177.
6. Townsend, A., Öhlén, C., Bastin, J., Ljunggren, H.-G., Foster, L. & Kärre, K. (1989) *Nature (London)* **340**, 443–448.
7. Braciale, T. J., Morrison, L. A., Swetser, M. T., Sambrook, J., Gething, M. J. & Braciale, V. L. (1987) *Immunol. Rev.* **98**, 95–114.
8. Harding, C. V. & Unanue, E. R. (1990) *Nature (London)* **346**, 574–576.
9. Miller, J. F. A., Morahan, G., Allison, J. & Hoffmann, M. (1991) *Immunol. Rev.* **122**, 103–116.
10. Kisielow, P., Bluthmann, H., Staerz, V. D., Steinmetz, M. & von Boehmer, H. (1988) *Nature (London)* **333**, 742–746.
11. Sha, W. C., Nelson, C. A., Newberry, R. D., Kranz, D. M., Russell, J. H. & Loh, D. (1988) *Nature (London)* **336**, 73–76.
12. Berg, L. J., Pullen, A. M., Fazekas de St Groth, B., Mathis, D., Benoist, C. & Davis, M. M. (1989) *Cell* **58**, 1035–1046.
13. Pircher, H., Burki, K., Lang, R., Hengartner, H. & Zinkernagel, R. M. (1989) *Nature (London)* **342**, 559–561.
14. Murphy, K. M., Heimberger, A. B. & Loh, D. Y. (1990) *Science* **250**, 1720–1722.
15. Townsend, A. R. M., McMichael, A. J., Carter, N. P., Huddleston, J. A. & Brownlee, G. G. (1984) *Cell* **39**, 13–25.
16. Townsend, A. R. M., Rothbard, J., Gotch, F. M., Bahadur, G., Wraith, D. & McMichael, A. J. (1986) *Cell* **44**, 959–968.
17. Palmer, M. S., Bentley, A., Gould, K. & Townsend, A. R. M. (1989) *Nucleic Acids Res.* **17**, 2353.
18. Greaves, D. R., Wilson, F., Lang, G. & Kioussis, D. (1989) *Cell* **56**, 979–986.
19. Lang, G., Wotton, D., Owen, M. J., Sewell, W. A., Brown, M. H., Mason, D. Y., Crumpton, M. J. & Kioussis, D. (1988) *EMBO J.* **7**, 1675–1682.
20. Tomonari, K. & Lovering, E. (1988) *Immunogenetics* **28**, 445–451.
21. Cerundolo, V., Elliott, T., Elvin, J., Bastin, J., Rammensee, H.-G. & Townsend, A. (1991) *Eur. J. Immunol.* **21**, 2069–2077.
22. Pircher, H., Hoffmann Rohrer, U., Moskophidis, D., Zingernagel, R. M. & Hengartner, H. (1991) *Nature (London)* **351**, 482–485.
23. Vasquez, N. J., Kaye, J. & Hendrick, M. (1992) *J. Exp. Med.* **175**, 1307–1316.
24. Rotzschke, O., Falk, K., Deres, K., Shild, H., Norda, M., Metzger, J., Jung, G. & Rammensee, H. G. (1990) *Nature (London)* **348**, 252–254.
25. Robbins, P. A. & McMichael, A. J. (1991) *J. Exp. Med.* **173**, 221–230.
26. Teh, H.-S., Kishi, H., Scott, B. & von Boehmer, H. (1989) *J. Exp. Med.* **169**, 795–806.
27. Blackman, M. A., Burgert, H. G., Woodland, D. L., Palmer, E., Kappler, J. W. & Marrack, P. (1990) *Nature (London)* **345**, 540–542.
28. Webb, S., Morris, C. & Sprent, J. (1990) *Cell* **63**, 1249–1256.
29. Schönrich, G., Kalinke, U., Momburg, F., Malissen, M., Schmitt-Verhulst, A.-M., Malissen, B., Hämmerling, G. J. & Arnold, B. (1991) *Cell* **65**, 293–304.
30. Dresser, D. W. (1962) *Immunology* **5**, 378–388.