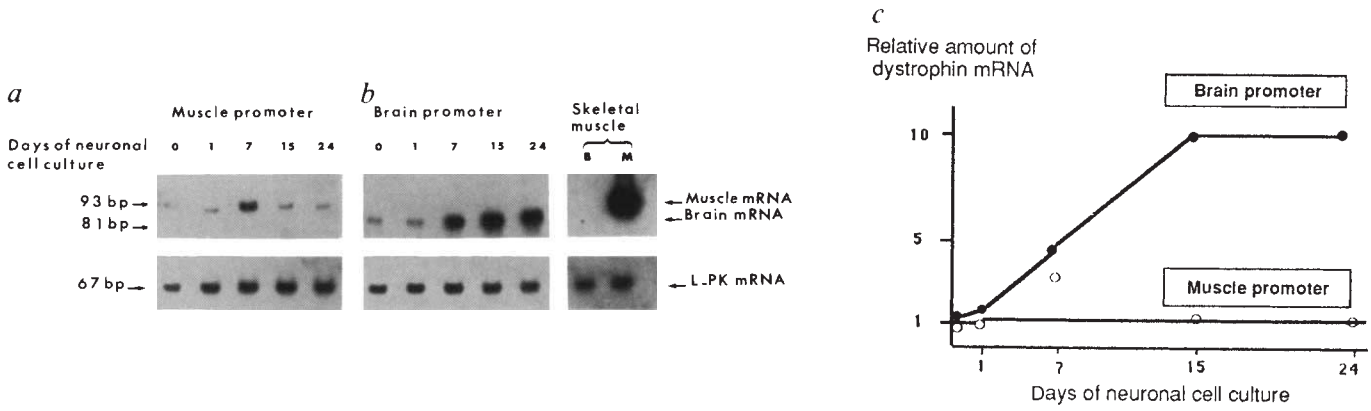

FIG. 2 Study of the brain-type and muscle-type dystrophin transcripts during neuronal maturation. *a*, Southern blots of PCR-amplified muscle-type transcripts (15 cycles) from mouse cultured neurons (top) co-amplified with L-PK transcripts used as internal standard (bottom). *b*, Southern blots of PCR-amplified brain-type transcripts (15 cycles) from mouse cultured neurons (top) co-amplified with L-PK transcripts used as internal standard (bottom). *c*, Relative amounts of brain-type and muscle-type transcripts of the dystrophin gene as a function of time of neuronal maturation. Methods are described in the legend to Fig. 1.

in glial cells, as in skeletal muscle, only the muscle promoter is used. In neurons, the brain-type mRNA is more abundant than the muscle-type mRNA, whereas it was not detected in either glial cells or skeletal muscle. After 30 cycles of amplification, however, minute amounts of transcript driven by the brain-type promoter are detectable in muscle and glial cells (Fig. 1*c*).

We also investigated the relative amounts of brain-specific and muscle-specific expression of the dystrophin mRNA in the course of *in vitro* maturation of neuronal cells from fetal mouse brain (see Fig. 2 legend). Figure 2 shows that the amount of brain-specific transcript increases steadily, reaching a plateau at day 14, with a total increment of ~10. By contrast, the muscle-specific transcript does not vary significantly, and after the fifteenth day of culture represents ~10% of the brain-specific transcript (Fig. 2).

Second to myogenic tissues, the central nervous system has the highest level of expression of the dystrophin gene, demonstrated by the levels of both mRNA[8,9] and protein[10]. It has already been suggested that in brain the dystrophin expression is neuronal in origin[10], and, in primary cultures of mouse brain cells, we find it is greater in neuronal than in glial cells[4]. As transcription of the first exon of brain dystrophin is driven by a specific promoter[2,3], we attempted to define the type of promoter used in neuronal and glial cells. Our results indicate that the brain promoter is active only in neurons, whereas the muscle promoter is used in skeletal muscle, in glial and in neuronal cells. A weak contamination by astrocytes (~1-5%), might contribute to the finding of some muscle-type transcript in cultured neurons. The muscle-type promoter is also active in cardiac and smooth muscle (data not shown). The trace amounts of brain-promoted transcripts that we find in muscle and glial cells after 30 cycles are probably attributable to illegitimate transcription[11] and are comparable to the levels of muscle-type transcript that we detect in cultured fibroblasts (Fig. 1*c*). We believe that this would account for an earlier discovery[3] of brain-specific transcript in muscle. In every cell type that we investigated, apart from neurons, the muscle promoter is stronger and more leaky than the brain promoter. This could explain why the illegitimate transcript found in fibroblasts is mainly transcribed from the muscle-type promoter. The increase that we observed during maturation of neurons in culture and in developing mouse brain (data not shown) indicates that brain-type dystrophin mRNA and protein could be neuron-specific developmental markers, like the voltage-gated sodium channel[12] and tubulin[13]. The subcellular location and function of dystrophin in neurons must be determined before any relation can be established between expression of this protein in brain and the mental retardation found in some Duchenne muscular dystrophy patients. □

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1. Monaco, A. P. & Kunkel, L. M. *Adv. Hum. Genet.* **17,** 61-98 (1988).
2. Nudel, U., Zuk, D., Zeelon, E., Levy Z., Neuman, S. & Yaffe, D. *Nature* **337,** 76-78 (1989).
3. Feener, C. A., Koenig, M. & Kunkel, L. M. *Nature* **338,** 509-511 (1989).
4. Chelly, J. *et al. Eur. J. Biochem.* (in the press).
5. Chelly, J., Kaplan, J. C., Maire, P., Gautron, S. & Kahn, A. *Nature* **333,** 858-860 (1988).
6. Berwald-Netter, Y., Martin-Moutot, N., Koulakoff, A. & Couraud, F. *Proc. natn. Acad. Sci. U.S.A.* **78,** 1245-1249 (1981).
7. Nowak, L., Ascher, P. & Berwald-Netter, Y. *J. Neurosci.* **7,** 101-109 (1987).
8. Nudel, U., Robzyk, K. & Yaffe, D. *Nature* **331,** 635-638 (1988).
9. Chamberlain, S. C. *et al. Science* **239,** 1416-1418 (1988).
10. Hoffman, E. P., Hudecki, M. S., Rosenberg, P. A., Pollina, C. M. & Kunkel, L. *Neuron* **1,** 411-420 (1988).
11. Chelly, J., Concordet, J. P., Kaplan, J. C. & Kahn, A. *Proc. natn. Acad. Sci. U.S.A.* **86,** 2617-2621 (1989).
12. Couraud, F., Martin-Moutoy, N., Koulakoff, A. & Berwald-Netter, Y. *J. Neurosci.* **6,** 192-198 (1986).
13. Eddé, B. *et al. Biol. Cell* **65,** 109-117 (1989).

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Role of self-peptides in positively selecting the T-cell repertoire

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THE fate of an immature thymocyte is determined by the specificity of its αβ T-cell receptor. Only cells expressing receptors that interact with sufficient affinity with major histocompatibility complex (MHC) molecules expressed on thymus epithelial cells are positively selected and go on to mature and seed the peripheral lymphoid organs[1–4]. The H–2K^b class-I MHC molecule positively selects for the maturation of cytotoxic T lymphocytes that will respond in the periphery to H–2K^b cells presenting a foreign peptide. We have now analysed the ability of variant H–2K^b molecules to positively select T-cells that respond to H–2K^b with ovalbumin. Our results indicate that self-peptides, presented in the groove of the class-I molecule on thymus epithelial cells, are critically involved in positive selection of the T-cell repertoire. Furthermore, the ability of four different H–2K^b variants to select this response in the thymus correlates with their ability to present the ovalbumin peptide, indicating that a self-peptide mimic of the foreign peptide could be involved in positive selection.

The structure of a class-I MHC molecule reveals a likely peptide-binding groove composed of a 'floor' of antiparallel β-stands and 'walls' of two long α-helices[5]. It has been proposed that during MHC-restricted recognition of foreign peptide the T-cell receptor makes contact with the outer face of the α-helices and the peptide lying between them[6-8]. Because the selection of the T-cell repertoire occurs during T-cell differentiation in the thymus in the absence of foreign peptide, we speculated that the outer face of the α-helices would be particularly important in positive selection and the peptide-binding groove would exert less influence. To determine whether this is so, we compared the ability of several H-2K^b variant MHC molecules to select a H-2K^b -restricted repertoire. We were most interested in two H-2K^b mutants, H-2K^{bm8} (changes at positions 22, 23, 24 and 30) and H-2K^{bm5} (changes at position 116), with substitutions only in the β-strands forming the floor of the groove[9,10]. These changes are probably not detectable on the exterior of the molecule, and in fact these variants are serologically indistinguishable from wild-type H-2K^b with many antibodies[9,11]. Their effects on T-cell recognition are likely to be due to changes in peptide binding[10]. Comparison of the structure of HLA-A2 with that of a second class-I molecule, HLA-Aw68, with 10 residue differences in positions lining the floor and walls of the groove, shows that each substitution causes only very local structural changes[12]. The backbone structure of the two molecules is the same, and unaltered residues remain essentially unperturbed. Two other H-2K^b mutants, H-2K^{bm1} (changes at positions 152, 155 and 156) and H-2K^{bm3} (changes of positions 77 and 89), have substitutions on the α-helices that locally affect serological sites[9] as well as point into the groove and affect peptide presentation[10].

C57BL/6 (B6, H-2^b) mice are able to mount an ovalbumin (OVA)-specific cytotoxic T lymphocyte (CTL) response when they are immunized with syngeneic cells transfected with an OVA vector or with spleen cells carrying OVA[13]. All the CTL activity is restricted by the H-2K^b class-I molecule and specific for the $OVA_{253-276}$ region (containing residues 253-276 of OVA). The response is heterogeneous, because analysis of clones and long-term lines has shown that T-cell receptor Vβ5, Vβ8 as well as other Vβ segments, are represented in the CTL response. To study the influence of changes in the class-I molecule on the selection of the H-2K^b-restricted repertoire, we injected bone marrow cells from (B6.PL-Thy-1^a × H-2K^b-mutant)F$_1$ mice into lethally irradiated B6 or H-2K^b-mutant mice. After 8-12 weeks, when bone marrow-derived T-cells had matured in the irradiated hosts, we primed the mice with OVA on H-2^b cells. We boosted spleen cells from these mice *in vitro* with an EL4 transfectant synthesizing OVA (E.G7-OVA) or with allogeneic H-2^d spleen cells, and measured CTL avtivity 5 days later. We typed the H-2^d-specific effector cells for their expression of Thy-1.1 (indicating bone-marrow origin) or Thy-1.2 only (indicating host origin). Cells that matured in an irradiated H-2K^{bm1} or H-2K^{bm8} host were not able to respond to H-2K^b with OVA (Table 1). By contrast, T-cells that matured in a wild-type H-2K^b, H-2K^{bm3} or H-2K^{bm5} host responded to OVA measured as the specific lysis of E.G7-OVA target cells. Spleen cells from all groups could respond to H-2^d cells in primary mixed lymphocyte culture to generate CTL that lyse P815 (H-2^d) target cells and 70-92% of this alloreactive CTL activity was sensitive to anti-Thy-1.1 antibody and complement, and therefore due to CTL of bone marrow origin. The failure of the [B6.PL × bm8 → bm8] chimaeras to respond to OVA implies that peptide is important in repertoire selection.

TABLE 1 Ability of H-2K^b-mutant MHC molecules to select the CTL response to OVA assayed in [F$_1$ → parent] radiation chimaeras

Radiation chimaeras	OVA-specific response: % Specific lysis of targets EL4	OVA-specific response: % Specific lysis of targets E.G7	Alloreactive response: % Specific lysis of targets EL4	Alloreactive response: % Specific lysis of targets P815	Alloreactive response: % Donor* T-cells
Expt 1					
(B6.PL × bm1)F$_1$ → bm1	3/0	0/1	4/2	48/30	92
(B6.PL × bm3)F$_1$ → bm3	10/6	41/27	2/0	41/18	91
(B6.PL × bm5)F$_1$ → bm5	8/4	37/27	7/4	47/24	83
(B6.PL × bm8)F$_1$ → bm8	6/3	10/10	6/3	43/24	74
Expt 2					
(B6.PL × bm5)F$_1$ → B6	8/3	56/47	3/4	70/49	83
(B6.PL × bm5)F$_1$ → bm5	7/4	56/42	6/3	77/58	85
(B6.PL × bm8)F$_1$ → B6	2/3	58/54	4/2	65/47	78
(B6.PL × bm8)F$_1$ → bm8	10/6	7/8	6/4	69/54	70

Radiation chimaeras were made by injecting 10^7 F$_1$ bone marrow cells depleted of T-cells by antibody-with-complement treatment into lethally irradiated (950 rad) female recipients of the indicated type, [F$_1$ bone marrow → irradiated host][1,2]. The chimaeras were maintained on antibiotic water and were primed after 8-12 weeks by intravenous injection of 2.5×10^7 irradiated spleen cells containing OVA as described previously[22]. In expt 1, spleen cells syngeneic with the marrow donor were used to prime, and in expt 2, B6.PL spleen cells were used. Seven days after priming, spleen cells from the chimaeras were stimulated in culture with irradiated (12,000 rad) E.G7-OVA cells[1,3] to boost the OVA-specific response, or with irradiated (3,000 rad) BALB/c (H-2^d) spleen cells to induce an alloreactive response. After 5 days in culture, CTL activity was assayed in a 3-hr ^{51}Cr-release assay. Percentage specific lysis at two effector-to-target ratios are shown: 6:1 and 2:1 (expt 1) and 100:1 and 33:1 (expt 2).

* CTL activity from the cultures stimulated with allogeneic BALB/c (H-2^d) spleen cells assayed on P815 (H-2^d) targets was typed for sensitivity to anti-Thy-1.1 antibody (19E12)[23] and anti-Thy-1.2 antibody (13.4)[24] with complement as described to determine the fraction of donor-derived (Thy-1.1 × Thy-1.2) effector cells. In brief, effector cells were treated with complement alone or with one or both antibodies with complement for 45 min at 37 °C before assaying on ^{51}Cr-P815 target cells. Controls with Thy-1.1 and Thy-1.2 CTL from B6.PL (Thy-1.1) and B6 (Thy-1.2) mice showed that both antibodies were Thy-1 allele-specific in their activity.

The nonresponsiveness of the [B6.PL × bm8 → bm8] and [B6.PL × bm1 → bm1] chimaeras could possibly be due to tolerance. This is unlikely because, as shown in Table 2, (B6.PL × bm8)F$_1$ and (B6.PL × bm1)F$_1$ mice responded to OVA with H-2K^b. But the homozygous mutant mice B6.C-H-2^{bm1} and B6.C-H-2^{bm8} are themselves nonresponsive to OVA. The other

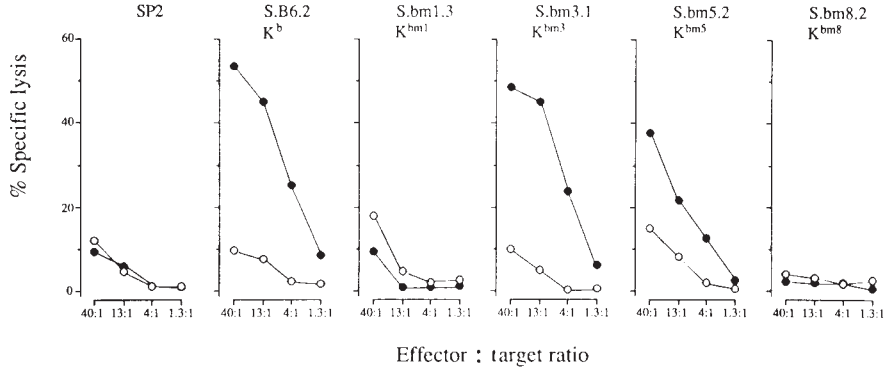

FIG. 1 Presentation of the $OVA_{253-276}$ peptide to B6 CTL by cells expressing H-2K^b-mutant molecules. Spleen cells from B6 mice that had been primed with OVA on syngeneic cells were boosted in culture for 5 days with irradiated E.G7-OVA cells and assayed for lysis of various targets that had been pulsed with medium or with the $OVA_{253-276}$ peptide. Target cells were SP2 (BALB/c, H-2^d myeloma) or hybrids formed by polyethylene glycol-mediated fusion of SP2 with spleen-derived lipopolysaccharide blasts from B6 or H-2K^b-mutant mice. S.B6.2 is a hybrid with B6; S.bm1.3 is a hybrid with bm1, and so on. The hybrid lines all express H-2D^b and the expected wild-type or mutant H-2K^b molecule as shown by serological and T-cell typing. Target cells were incubated with synthetic $OVA_{253-276}$ peptide, 100 μg ml^{-1} for 1 h at 37 °C and washed before the 3-hr ^{51}Cr-release assay. ●, $OVA_{253-276}$; ○, no antigen.

TABLE 2 Failure to respond to OVA in the [F_1 → parent] radiation chimaeras is not due to tolerance

	Responder	% Specific lysis on targets: E.G7-OVA	EL4
Expt 1			
	B6	60/41	17/9
	(B6 × bm1)F_1	47/30	18/7
	(B6 × bm3)F_1	66/53	25/8
	(B6 × bm5)F_1	58/46	14/6
	(B6 × bm8)F_1	53/36	9/8
Expt 2			
	B6	60/53	0/0
	bm1	4/2	2/2
	bm3	41/25	3/0
	bm5	54/39	3/1
	bm8	12/6	23/15

The indicated responder mice were immunized by intravenous injection of 2.5×10^7 irradiated (1,000 rad) syngeneic spleen cells that had been 'loaded' with OVA[13]. Seven days later, spleen cells from the primed animals were re-stimulated in culture with syngeneic spleen cells that had been incubated with a CNBr-digest of OVA, 100 μg ml^{-1}, and washed. The CNBr fragments of OVA include the H-$2K^b$-restricted targeting peptide. CTL activity was measured 5 days later and is expressed as % specific lysis at effector-to-target ratios of 50:1 and 17:1. The ability of the CTL to lyse E.G7-OVA targets correlated with their ability to lyse syngeneic H-$2K^b$-mutant targets in the presence of the OVA peptide.

TABLE 3 [F_1 → parent] radiation chimaeras contain H-$2K^b$-expressing antigen-presenting cells

Responder spleen cells	Stimulator spleen cells	Anti-H-$2K^b$-specific response (% specific ^{51}Cr release) P815	EL4
bm1	B6	13/4	58/37
	(B6.PL × bm1)F_1	16/6	64/48
	(B6.PL × bm1)F_1 → bm1	12/8	55/39
bm8	B6	10/1	67/62
	(B6.PL × bm8)F_1	8/1	64/47
	(B6.PL × bm8)F_1 → bm8	8/3	67/47

Responder spleen cells from naive B6.C-H-2^{bm1} (bm1) or B6.C-H-2^{bm8} (bm8) mice were cultured *in vitro* for 5 days with an equal number of 3,000 rad spleen cells from stimulator mice as shown. CTL activity was assayed 5 days later on P815 (H-2^d) and EL4 (H-2^b) target cells at effector-to-target ratios of 48:1 and 16:1.

mutant mice, B6.C-H-2^{bm3} and B6.C-H-2^{bm5}, mounted a CTL response to OVA which cross-reacts on OVA with H-$2K^b$ (Table 2). Therefore we saw a correlation between the ability of four H-$2K^b$ mutants to make a CTL response to OVA and their ability to select the OVA-with-H-$2K^b$ repertoire in radiation chimaeras. To study this more closely, we analysed the ability of the H-$2K^b$ variants to present the H-$2K^b$ restricted OVA peptide *in vitro*. A heterogeneous CTL line from B6 mice specific for H-$2K^b$ with $OVA_{253-276}$ was tested for lysis of target cells expressing different class-I molecules. CTL did not lyse SP2 (H-2^d) cells in the presence or absence of the OVA peptide, but a hybrid cell line, S.B6.2 (SP2 fused with B6 cells), was lysed in a peptide-specific way (Fig. 1). Target cells expressing H-$2K^{bm1}$ or H-$2K^{bm8}$ however, were not recognized by this CTL line in the presence of peptide, whereas H-$2K^{bm3}$ and H-$2K^{bm5}$ cells were peptide-specific targets. In other experiments we have generated eight independent CTL clones from B6 mice specific for H-$2K^b$ with $OVA_{253-276}$, none of which recognizes H-$2K^{bm1}$ or H-$2K^{bm8}$ presenting cells.

The inability of cells derived from H-$2K^{bm1}$ or H-$2K^{bm8}$ mice to present the OVA peptide indicates a trivial explanation for the failure of such mice to select the response in the [F_1 → parent] chimaeras. So, although we typed the alloreactive CTL as >70% of bone-marrow donor origin, the cells in the chimaeras presenting OVA to resting CTL could still have been of host origin. If this were the case, then [B6.PL × bm1 → bm1] and [dB6.PL × bm8 → bm8] chimaeras would have inappropriate presenting cells for the H-$2K^b$-with-OVA response. This explanation is unlikely to be true for two reasons. First, previous work indicates that antigen-presenting cells turn over rapidly after irradiation[14], and second, we always injected the mice with immunogenic cells expressing H-$2K^b$. Nevertheless, we tested the hypothesis by assaying the ability of spleen cells from these chimaeras to stimulate anti-H-$2K^b$ CTL. Splenocytes from [B6.PL × bm1 → bm1] and [B6.PL × bm8 → bm8] radiation chimaeras were as effective in stimulating an H-$2K^b$-specific CTL response as were cells from normal F_1 animals (Table 3). We conclude that the nonresponsive chimaeras had appropriate H-$2K^b$-expressing presenting cells.

The H-$2K^{bm8}$ molecule differs from wild-type H-$2K^b$ in three positions in a β-strand in the floor of the peptide-binding site. Two of these, at positions 22 and 24, point up into the site, whereas position 23 points down[10]. The inability of H-$2K^{bm8}$ to select the heterogeneous response to H-$2K^b$ with $OVA_{253-276}$ indicates very strongly that self-peptides associated with the class-I molecules of thymic epithelial cells play a critical part in selecting the T-cell repertoire. The ability of variant H-$2K^b$ molecules to select is antigen-specific, because [B6.PL × bm8 → bm8] chimaeras are responders to VSV nucleocapsid protein with H-$2K^b$, meaning that H-$2K^{bm8}$ does select for this H-$2K^b$-restricted response (data not shown). The idea that tissue-specific self-peptides[15,16] or erronous self-peptides[17] are involved in positive selection has been suggested previously, and in some cases background genes (that is, not the restriction element) influence selection[18–20]. How the correlation that we observed between the ability of class-I molecules to present a foreign peptide and its ability to select the appropriate repertoire in the thymus comes about is difficult to understand and may not strictly apply for all antigens[21]. The restriction element would be expected to select sites on the antigen which intereact with the MHC (agretopes), whereas the T-cell receptor would be expected to interact with other regions of the antigen (epitopes). But T-cells that respond to a foreign peptide binding only to one side of the class-I groove and obscuring that part of the MHC molecule, may have to make many important receptor contacts with the opposite side of the MHC molecule. Thymic selection of such a T-cell receptor repertoire would depend on thymic MHC presenting peptides in a similar way. □

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1. Bevan, M. J. *Nature* **269,** 417–418 (1977).
2. Zinkernagel, R. M. *et al. J. exp. Med.* **147,** 882–896 (1978).
3. Kisielow, P. *et al. Nature* **335,** 730–733 (1988).
4. Sha, W. C. *et al. Nature* **336,** 73–76 (1988).
5. Bjorkman, P. J. *et al. Nature* **329,** 506–512 (1987).
6. Davis, M. M. & Bjorkman, P. J. *Nature* **334,** 395–402 (1988).
7. Claverie, J.-M., Prochnicka-Chalufour, A. & Bougueleret, L. *Immun. Today* **10,** 10–14 (1989).
8. Chothia, C., Boswell, D. R. & Lesk, A. M. *EMBO J.* **7,** 3745–3755 (1988).
9. Nathenson, S. G. *et al. A. Rev. Immun.* **4,** 471–502 (1986).
10. Bjorkman, P. J. *et al. Nature* **329,** 512–518 (1987).
11. Bluestone, J. A. *et al. J. Immun.* **137,** 1244–1250 (1986).
12. Garrett, T. P. J. *et al. Nature* **342,** 692–696 (1989).
13. Moore, M. W., Carbone, F. R. & Bevan, M. J. *Cell* **54,** 777–785 (1988).
14. Ron, Y., Lo, D. & Sprent, J. *J. Immun.* **137,** 1764–1771 (1986).
15. Marrack, P. & Kappler, J. *Immun. Today.* **9,** 308–315 (1988).
16. Marrack, P., McCormack, J. & Kappler, J. *Nature* **338,** 503–505 (1989).
17. Kourilsky, P. & Claverie, J.-M. *Cell* **56,** 327–329 (1989).
18. Ogata, M. *et al. J. Immun.* **136,** 1178–1185 (1986).
19. Singer, A. *et al. Prog. Immun.* **VI,** 60–66 (1986).
20. Fry, A. M., Cotterman, M. M. & Matis, L. A. *J. Immun.* **143,** 2723–2729 (1989).
21. Kast, W. M., deWaal, L. P. & Meleif, C. J. M. *J. exp. Med.* **160,** 1752–1766 (1984).
22. Carbone, F. R. & Bevan, M. J. *J. exp. Med.* (in the press).
23. Bernstein, I. D., Tam, M. R. & Nowinski, R. G. *Science* **207,** 68–71 (1980).
24. Marshak-Rothstein, A. *et al. J. Immun.* **122,** 2491–2497 (1979).

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