Proc. Natl. Acad. Sci. USA
Vol. 88, pp. 613–616, January 1991
Immunology

Direct binding of secreted T-cell receptor β chain to superantigen associated with class II major histocompatibility complex protein

(T-cell recognition/staphylococcal enterotoxin)

NICHOLAS R. J. GASCOIGNE* AND KRISTINA T. AMES

Department of Immunology, Research Institute of Scripps Clinic, La Jolla, CA 92037

Communicated by Frank J. Dixon, October 22, 1990

ABSTRACT **The interaction of the T-cell receptor (TCR) with peptide antigen plus major histocompatibility complex (MHC) protein requires both α and β chains of the TCR. The "superantigens" are a group of molecules that are recognized in association with MHC class II but that do not appear to conform to this pattern. Superantigens are defined as such because they cause the activation or thymic deletion of many or all T cells bearing specific TCR β-chain variable region (V_β) elements. The strong association of particular V_βs with T-cell responses to superantigens suggests that their interaction with the TCR is fundamentally different from that of most antigens. We have directly investigated the involvement of the β chain in recognition of a superantigen by using a secreted, truncated TCR β chain and the bacterial superantigen staphylococcal enterotoxin A complexed to cell-surface MHC class II. We demonstrate that this interaction is specific for the enterotoxin and is dependent on MHC class II expression by the cell. The reaction can be inhibited by antibodies against the three components of the reaction: V_β, enterotoxin, and class II. This shows that the TCR β chain is sufficient to mediate the interaction with a superantigen–class II complex. The TCR α chain and co-receptors such as CD4 are not required.**

T-cell recognition of antigen plus major histocompatibility complex (MHC) proteins requires the specificity of both α and β chains of the T-cell receptor (TCR). Small changes in the structure of the hypervariable regions of the TCR chains can have dramatic influences on reactivity to MHC and antigen (1). The "superantigens" (2) or "co-tolerogens" (3) are a class of TCR ligands that cause almost complete deletion or activation of T cells expressing certain β chain variable regions (V_β) (reviewed in ref. 4). These antigens include endogenous molecules such as the minor lymphocyte-stimulating (Mls) gene products and other undefined molecules (4). There is also a group of microbial toxins including the staphylococcal enterotoxins (SEA, SEB, etc.) that have similar effects (4). The constitution of the α chain and the hypervariable region at the VDJ_β junction (D, diversity; J, joining) has no detectable influence on the specificity. For example, Mls-$2^a/3^a$ causes deletion or activation of T cells bearing $V_\beta3$ (5–7). As an example of the microbial toxins, SEA activates cells bearing $V_\beta1$, -3, -11, and -12 (8, 9). No superantigen-like effect has been noted for TCR V_αs. These do not seem to be subject to the massive deletions common to many V_βs (10–12). All of the superantigens require MHC class II expression to be recognized by T cells (2–4, 13, 14). In the case of the microbial toxins, this is because they bind strongly and specifically to the class II molecule (15–17). Of all the superantigens, only the SEs and some related molecules are defined proteins.

Modeling of the TCR by using antibody structures suggests that both α and β chains of the TCR complex should be involved in the interaction with antigen plus MHC (18–20). The special relationship between the V_β and the superantigen raises the possibility that the β chain might break these rules and interact directly with the superantigen–class II complex. This is supported by recent experiments showing the importance of the fourth hypervariable region of V_β in superantigen recognition (21, 22). To test whether the β chain is sufficient for the interaction we have used a soluble TCR β chain protein expressed from a truncated gene lacking a transmembrane segment (23). The β chains are secreted from transfected lymphoid cells. They are stable and appear to be in a native conformation in that they are recognized by appropriate anti-TCR antibodies (23). Using a cell-binding assay, we show here that the isolated β chains interact specifically with a superantigen (SEA) complexed to MHC class II molecules. This interaction is dependent on class II expression and is inhibited by antibodies against each of the components of the reaction. Therefore, the TCR β chain, in the absence of α chain and CD4, is sufficient to mediate recognition of a superantigen: MHC class II complex of sufficient strength to bind cells.

MATERIALS AND METHODS

Antibodies. H57-597 (24) was a gift of R. Kubo (National Jewish Center, Denver); it recognizes a determinant on the β chain of the murine TCR (23). KJ25 is specific for $V_\beta3$ (5); it was a gift of A. Pullen and P. Marrack (National Jewish Center, Denver). 1.F2 recognizes a determinant on $V_\alpha11$ (12). These three antibodies are hamster IgGs. EC-A1 recognizes SEA (25); it was a generous gift of S. Kaveri (IDEC Pharmaceuticals, La Jolla, CA). A2B4.2 is specific for the V_α of 2B4 (26, 27) and was a gift of L. Samelson (National Institutes of Health, Bethesda, MD). Q5/13 is specific for a monomorphic determinant on HLA-DR, -DP, and -DQ (28). W6/32 recognizes a monomorphic determinant on HLA-A, -B, and -C (29). Q5/13 and W6/32 were gifts of V. Quaranta (Scripps Clinic). Antibodies of this group are mouse IgG2a. All antibodies were purified from ascites by using protein A-Sepharose (Pharmacia LKB).

Cells. Raji cells were used in most cell-binding experiments. Raji is a Burkitt lymphoma cell that expresses MHC classes I and II and is a very effective presenter of enterotoxins (16). RM3 is a derivative of Raji that has lost class II expression (30). It was generously provided by M. Peterlin (University of California, San Francisco). Daudi is another Burkitt lymphoma cell. It does not express β_2-microglobulin and thus has no cell-surface class I (31). All cells were maintained in RPMI 1640 medium supplemented with 10%

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: TCR, T-cell receptor; V_β, β-chain variable region; V_α, α-chain V region; MHC, major histocompatibility complex; SE, staphylococcal enterotoxin; D, diversity; J, joining; C, constant.

*To whom reprint requests should be addressed.

fetal calf serum, L-glutamine, 50 μM 2-mercaptoethanol, and antibiotics.

Soluble β Chain. TCR β chain was produced as described (23). A gene encoding a truncated form of the β chain from the pigeon cytochrome *c* I-E^k-specific T cell 2B4 (26) was expressed in the myeloma cell line J558L. The 2B4 β chain consists of $V_\beta3$, $D_\beta2$, $J_\beta2.5$, and $C_\beta2$ (C, constant) (32). The truncated molecule has a stop codon within the $C_\beta2.2$ exon, resulting in a protein lacking a transmembrane region (23). This protein is secreted, is stable, and reacts with a number of anti-TCR antibodies (23). The β chain was partially purified for use in cell-binding assays. Culture supernatants were precipitated with 60% saturated ammonium sulfate. The precipitate was dialyzed extensively against phosphate-buffered saline (PBS; pH 7.3) and affinity purified by using a column of the antibody H57-597 coupled to Affi-Gel 10 (Bio-Rad).

Cell-Binding Assay. The anti-β-chain monoclonal antibody H57-597 (24) (50 μg/ml) was coated onto wells of "easy-wash"-modified ELISA plates (Corning) overnight at 4°C. The antibody was removed and the wells were blocked for nonspecific binding with bovine serum albumin (1 mg/ml) in PBS. Soluble 2B4 β chain was added (50 μl per well of 1–5 μg/ml) and allowed to bind for 2 hr at 37°C. The wells were washed with PBS and 10^5 metabolically labeled cells were added in PBS containing 5% fetal calf serum. Cells were labeled in methionine-free RPMI 1640 medium with [^{35}S]methionine (15 μCi per 10^6 cells; 1 Ci = 37 GBq) for 1 hr followed by three washes in PBS. SEA or SEB (Toxin Technology, Madison, WI) (90 μg/ml) was added during labeling. The plates were spun briefly at 200 × *g* and incubated at 37°C for 2 hr. The wells were washed gently with PBS with 5% fetal calf serum five times, after which 0.5% Nonidet P-40 in Tris-buffered saline was added to lyse the cells. The ^{35}S released from the bound cells was measured in a scintillation counter. The number of cells bound (±SD) was calculated from the specific activity of the labeled cells. Control wells using bovine serum albumin or antibodies such as W6/32 or Q5/13 bound to the plastic were used as positive and negative controls. Binding to the positive control antibodies was routinely 40–60% of the input 10^5 cells.

Flow Cytometry. Cells were treated with SEA (100 μg/ml) in RPMI 1640 medium for 45 min at 37°C. They were washed three times in Hanks' balanced salt solution containing 0.01% sodium azide plus 0.1% bovine serum albumin. They were stained with first-stage antibody (10–20 μg/ml) in this medium for 45 min at 4°C and washed as described above. The second-stage antibody [goat anti-mouse IgG (Sigma)] was added (1/20; vol/vol) and incubated as described above. The cells were washed again three times and analyzed in a FACS IV (Becton Dickinson).

RESULTS

Preliminary experiments looking for a direct interaction between the soluble β chain and SEA were not fruitful. No specific interaction was noted either in coprecipitation experiments with labeled β chain and SEA, with or without chemical cross-linking, or in a radioimmunoassay with labeled β chain and SEA adsorbed to plastic (data not shown). Cell-binding assays have in the past been used to demonstrate presumed low-affinity interactions—for example, the interaction between CD4 and MHC class II (33) and that between CD8 and class I (34). Such assays have the advantage of massively increasing the avidity of interaction. Since nothing is currently known of the affinity of the TCR for its ligands, we developed a system for binding labeled cells to immobilized, secreted, TCR β-chain protein. Initially, we used human class II-expressing cells to present SEA to mouse TCR β chain, since the *Staphylococcus aureus* enterotoxins are reported to bind better to human class II than to mouse class II (4). Mouse T cells are able to recognize SE on human cells (ref. 35; N.R.J.G., unpublished data).

The monoclonal antibody H57-597 (24) was allowed to adhere to microtiter wells. It reacts with a common determinant on TCR β chains, presumably the C region (23). These antibody-coated wells were then incubated with partially purified secreted β-chain protein. After appropriate washing steps, 1 × 10^5 metabolically radiolabeled cells (usually HLA class II-expressing Raji cells), either untreated or treated with SE, were added and allowed to interact with the β chains. The wells were washed to remove unbound cells and the remaining cells were lysed in detergent and enumerated by scintillation counting.

The truncated $V_\beta3^+$ β chain from the helper T-cell hybridoma 2B4 was used in these experiments. Table 1 shows the results from a comparison of binding of Raji cells treated with SEA to β chain attached to plastic via three different hamster antibodies specific for TCR molecules. H57-597 (24) is significantly more effective than KJ25, which recognizes $V_\beta3$ (5). The antibody 1.F2, which recognizes the $V_\alpha11$ chain expressed by 2B4 (12), does not support the binding. We have previously shown that KJ25 binds to the secreted 2B4 β chain (23). Thus, cell binding is dependent on the specificity of the antibody used as the first stage on the plastic well and also on the region of the β chain recognized by the antibody; an anti-V_β works poorly compared to a probable anti-C_β, presumably because the binding site for SEA–class II is masked by the anti-V_β antibody.

The 2B4 β chain consists of $V_\beta3$, $D_\beta2$, and $J_\beta2.5$ (32). $V_\beta3$-expressing T cells have been shown to be strongly activated by SEA, and to a lesser extent by SEB (8). We compared binding of SEA- and SEB-treated Raji cells to the 2B4 β chain. Fig. 1 demonstrates that the $V_\beta3$-containing truncated β chain binds to Raji cells bearing SEA but not to those bearing SEB. Since previous work by others showed that cells expressing $V_\beta3$ or $V_\beta8$ are activated by SEB (2, 8), the lack of binding of SEB-treated Raji cells was initially surprising. However, this is readily explained by an artefact in the original experiments caused by contamination of the SEB preparation with small quantities of SEA (J. Kappler, personal communication; see *Discussion*).

The activation of T cells by SE occurs only when the SE is complexed to MHC class II molecules (2, 4, 13, 14, 16, 17). We therefore tested the requirement for class II molecules in SEA-mediated binding of cells to TCR β chains. Binding of Raji cells was compared to binding of RM3 and Daudi cells in the presence or absence of SEA. Raji and Daudi are both Burkitt lymphoma cell lines. RM3 is a class II-negative mutant of Raji (30), and Daudi expresses class II molecules but is defective in class I expression (31). Flow cytometry analysis (Table 2) indicates that Daudi expresses ≈13% of the

Table 1. Specificity of first-stage antibody in cell-binding assay

	Antibody	β chain	Cells bound (±SD)
Exp. 1	H57-597	−	90 (21)
	H57-597	+	16,909 (1692)
	1.F2	−	67 (10)
	1.F2	+	340 (222)
Exp. 2	H57-597	+	9,250 (432)
	KJ25	−	137 (40)
	KJ25	+	2,262 (552)

The hamster anti-TCR antibodies H57-597 (24) (anti-TCR β-chain, probably anti-C region), 1.F2 (12) (anti-$V_\alpha11$), and KJ25 (5) (anti-$V_\beta3$) were compared for their ability to attach the secreted 2B4 β chain to plastic microtiter wells to support the attachment of Raji cells treated with SEA. The antibodies were added to the wells as 50 μl of a solution (50 μg/ml) and were allowed to bind for 16 hr at 4°C. Metabolically labeled, SEA-treated Raji cells were allowed to bind and were enumerated as described.

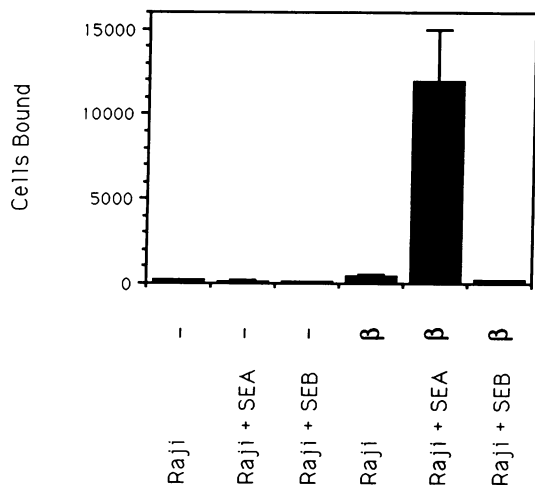

FIG. 1. Truncated TCR β chain binds to Raji plus SEA. Metabolically labeled Raji cells were treated with medium, SEA, or SEB and allowed to bind to $V_\beta3$-bearing truncated β chains in microtiter wells. The number of cells bound (±SD) was determined. Only Raji cells treated with SEA bound to the β chains. Antibodies recognizing class I (W6/32) and class II (Q5/13) were adsorbed to the plate and used as positive controls for cell binding. They bound 40,200 and 45,000 cells, respectively, of the 100,000 cells added. Thus, the 11,900 SEA-treated Raji cells that bound represented 26–30% of maximum binding.

level of Q5/13-reactive class II and <1% of the W6/32-reactive class I expressed by Raji. RM3 expresses <1% of class II and almost 3 times the amount of the class I expressed by Raji. The anti-SEA antibody EC-A1 shows that RM3 binds 1.5% and Daudi binds 22.5% of the amount of SEA bound by Raji. The cell-binding experiment shown in Fig. 2 demonstrates that only the SEA-treated cells expressing MHC class II molecules are able to bind to $V_\beta3$. The lack of MHC class I on Daudi does not stop the interaction, whereas the lack of class II on RM3 precludes binding. The number of Daudi cells bound via SEA to the β chain reflects the lower number of class II molecules on this cell line compared to Raji.

The specificity of the interaction was further determined by antibody blocking experiments (Fig. 3). The binding of SEA-treated Raji cells to the 2B4 β chain was measured in the presence of various amounts of antibodies recognizing MHC class I, class II, V_α, V_β, and SEA. This experiment demonstrates that antibodies directed to the presumed components of the interaction—MHC class II, SEA, and $V_\beta3$—all block cell binding. Antibodies against V_α, which recognizes nothing in the reaction, or against MHC class I, which binds to the Raji cells, do not block the interaction.

DISCUSSION

We have demonstrated that an isolated β chain of the TCR is sufficient to bind specifically to a SE superantigen complexed

Table 2. MHC expression and SEA binding by cell lines

Cells	Mean fluorescence		
	Q5/13	W6/32	EC-A1
Raji + SEA	934	446	262
RM3 + SEA	7	1218	4
Daudi + SEA	118	3	59

Raji, RM3, and Daudi cells were incubated with SEA (100 μg/ml) for 45 min at 37°C as described. They were washed and stained sequentially with the antibody shown followed by fluoresceinated goat anti-mouse IgG. The fluorescence intensity shown is the mean fluorescence minus the background obtained when no first-stage antibody was used. The mean fluorescence is presented in arbitrary units.

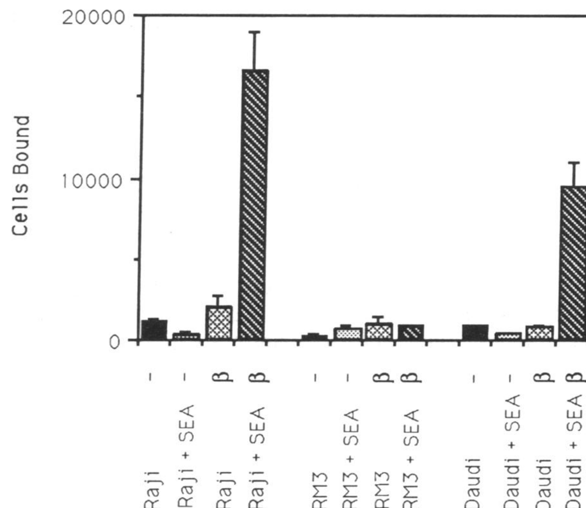

FIG. 2. Class II requirement for SEA–$V_\beta3$ interaction. Raji, RM3, and Daudi cells were compared for their ability to bind to truncated 2B4 β chains. All three are Burkitt lymphoma cells; Raji expresses MHC classes I and II, RM3 is a derivative of Raji that has lost class II expression, and Daudi does not express β_2-microglobulin and thus has no cell-surface class I (30, 31). Cell binding to antibodies adsorbed to 96-well plates showed that binding of Raji and SEA to β chain in this experiment represented 25.4% of binding to W6/32. For RM3 and SEA, this was 1.7%. Daudi and SEA binding to β chain was 24.1% of binding to the anti-class II antibody Q5/13 (data not shown). Methods are as described in Fig. 1.

to cell-surface MHC class II molecules. This indicates directly that the TCR β chain interacts with the SE–class II complex without an obligatory contribution from the α chain, even though an α chain contribution may sometimes be necessary (21). It also shows that co-receptors such as CD4 are not required for recognition. To our knowledge, interaction between an isolated TCR molecule and a natural ligand has not been reported previously.

In our experiments, the $V_\beta3$-bearing 2B4 β chain is specific for SEA and shows no interaction with SEB. This is at

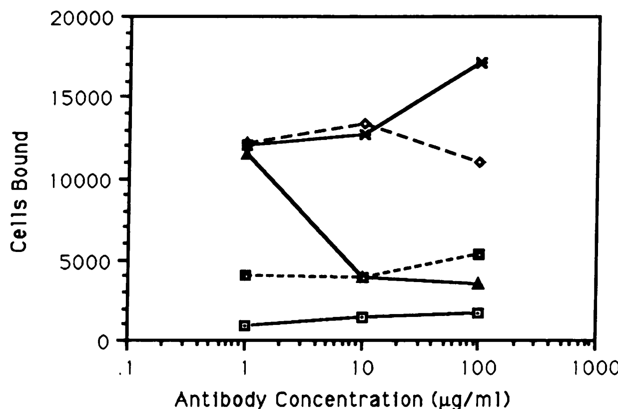

FIG. 3. Blocking of the SEA–$V_\beta3$ interaction by antibodies. SEA-treated Raji cells were allowed to interact with TCR β chain in the presence of various antibodies. Q5/13 (□) is specific for a monomorphic determinant on class II (28), EC-A1 (▲) is specific for SEA (25), KJ25 (■) is specific for $V_\beta3$ (5), A2B4.2 (×) is specific for the V_α of 2B4 (26, 27), and W6/32 (◇) is specific for a monomorphic determinant on HLA-A, -B, and -C (29). Methods are as described in Fig. 1. After metabolic and SEA labeling, and before washing, cells were incubated with 10% normal mouse serum (4°C; 30 min) in medium containing 0.01% sodium azide to block Fc receptors. Antibodies were added to the cell binding experiment at the indicated concentrations. Binding was performed in the presence of 0.01% azide.

variance with published reports of the V_β specificity of SEA and SEB. Although SEA is reported to activate cells expressing $V_\beta3$ and others (8, 9), SEB has also been described as activating $V_\beta3^+$ T cells, as well as those bearing $V_\beta7$ and -8 (2, 8). In addition, the 2B4 cell line has been shown to respond, albeit weakly, to SEB (8). There are a number of possible explanations for this anomaly. First, the $V_\beta3$ from 2B4 might simply react poorly to SEB, suggesting that there might be a J-region or α chain effect. Second, SEB has a lower relative affinity for class II than does SEA (15). There might not be sufficient SEB present on the cells to allow the binding to occur. This seems unlikely from flow cytometry with a monoclonal anti-SEB antibody (data not shown). Third, the reported SEB effect on $V_\beta3$ T cells might have been due to contamination with SEA. This is suggested by the fact that the quantity of SEB required for stimulation of $V_\beta3$-bearing cells is several orders of magnitude greater than that required for SEA stimulation (2, 8). This interpretation is probably correct because of the finding that recombinant SEB generated from a β-galactosidase fusion protein stimulates $V_\beta7$- and $V_\beta8$-expressing T cells but not those bearing $V_\beta3$ (J. Kappler, personal communication).

These data indicate that the secreted version of the 2B4 β chain retains each of the properties of the interaction of the complete TCR with the superantigen SEA. The interaction is enterotoxin specific. There is a requirement for SEA to be bound to MHC class II molecules for it to be recognized. Any other sites for binding of SEA onto the cell surface are not sufficient to enable the interaction to take place. Also, we have been unable to demonstrate an interaction between radiolabeled β chain and plate-bound or soluble SEA. Blocking of the V_β molecule, the MHC class II, or the SEA bound to the cell surface is enough to block the interaction between the SEA-expressing cell and the β chain.

The V_β recognizes a superantigen only when it is bound to class II, yet the class II seems to have little specificity in the interaction; human class II presents SE to mouse TCRs and class II presents SE to T cells whose receptors are class I restricted (35). These data make it likely that when it interacts with the SE–class II complex, the β chain either does not make contact with the class II molecule or it binds in a manner that has been conserved between MHC classes I and II and between species. In either case, the strength of the interaction between class II-bound enterotoxin and the β chain of the TCR is sufficient for cell binding. It is worth noting that although the method that we have used to demonstrate this interaction is designed to be able to measure a very weak interaction (since it greatly multiplies the avidity of the β chain), this may in fact mimic the natural condition since T cells are normally activated by antigens (super or otherwise) on cells. There is little information on the affinity of the TCR for antigen or MHC. A recent estimate of the number of ligated TCR molecules required for 50% maximal cell-mediated lysis of a target cell suggests that only $\approx$0.5% receptor occupancy is necessary (36). This translates into $\approx 10^2$ TCR molecules. Although this is only a small proportion of the available TCR molecules, it could still represent a vast functional avidity compared to the affinity of an individual TCR molecule.

We are grateful to Drs. Vito Quaranta, Pam Fink, and John Kappler for important suggestions; to Drs. S. Kaveri, R. Kubo, L. Samelson, P. Marrack, and V. Quaranta for antibodies; to Dr. M. Peterlin for RM3 cells; and to Drs. S. Webb, M. Irwin, and S. Jameson for critical reading of the manuscript. This is manuscript 6334-IMM from the Research Institute of Scripps Clinic, Department of Immunology. This work was supported by Grant GM-39476 from the National Institutes of Health, by the Concern Foundation for Cancer Research, and was aided by Basil O'Connor Starter Scholar Research Award Grant 5-674 through a grant from the Scott Paper "Helping Hand Fund" to the March of Dimes Birth Defects Foundation. N.R.J.G. is a Scholar of the Leukemia Society of America.

1. Matis, L. A. (1990) *Annu. Rev. Immunol.* **8,** 65–82.
2. White, J., Herman, A., Pullen, A. M., Kubo, R., Kappler, J. W. & Marrack, P. (1989) *Cell* **56,** 27–35.
3. Woodland, D., Happ, M. P., Bill, J. & Palmer, E. (1990) *Science* **247,** 964–967.
4. Marrack, P. & Kappler, J. (1990) *Science* **248,** 705–711.
5. Pullen, A. M., Marrack, P. & Kappler, J. W. (1988) *Nature (London)* **335,** 796–801.
6. Abe, R., Vaccio, M. S., Fox, B. & Hodes, R. J. (1988) *Nature (London)* **335,** 827–830.
7. Fry, A. M. & Matis, L. A. (1988) *Nature (London)* **335,** 830–832.
8. Callahan, J. E., Herman, A., Kappler, J. W. & Marrack, P. (1990) *J. Immunol.* **144,** 2473–2479.
9. Takimoto, H., Yoshikai, Y., Kishihara, K., Matsuzaki, G., Kuga, H., Otani, T. & Nomoto, K. (1990) *Eur. J. Immunol.* **20,** 617–621.
10. Bill, J., Appel, V. & Palmer, E. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 9184–9188.
11. Tomonari, K., Lovering, E., Fairchild, S. & Spencer, S. (1989) *Eur. J. Immunol.* **19,** 1131–1135.
12. Jameson, S. C., Kaye, J. & Gascoigne, N. R. J. (1990) *J. Immunol.* **145,** 1324–1331.
13. Fleischer, B. & Schrezenmeier, H. (1988) *J. Exp. Med.* **167,** 1697–1707.
14. Janeway, C. A., Jr., Yagi, J., Conrad, P. J., Katz, M. E., Jones, B., Vroegop, S. & Buxser, S. (1989) *Immunol. Rev.* **107,** 61–88.
15. Fraser, J. D. (1989) *Nature (London)* **339,** 221–223.
16. Fischer, H., Dohlsten, M., Lindvall, M., Sjogren, H.-O. & Carlsson, R. (1989) *J. Immunol.* **142,** 3151–3157.
17. Mollick, J. A., Cook, R. G. & Rich, R. R. (1989) *Science* **244,** 817–820.
18. Davis, M. M. & Bjorkman, P. J. (1988) *Nature (London)* **334,** 395–402.
19. Claverie, J.-M., Protchicka-Chalufour, A. & Bougueleret, L. (1989) *Immunol. Today* **10,** 10–14.
20. Chothia, C., Bothwell, D. R. & Lesk, A. M. (1988) *EMBO J.* **7,** 3745–3755.
21. Pullen, A. M., Wade, T., Marrack, P. & Kappler, J. W. (1990) *Cell* **61,** 1365–1374.
22. Choi, Y., Herman, A., DiGiusto, D., Wade, T., Marrack, P. & Kappler, J. (1990) *Nature (London)* **346,** 471–473.
23. Gascoigne, N. R. J. (1990) *J. Biol. Chem.* **265,** 9296–9301.
24. Kubo, R. T., Born, W., Kappler, J. W., Marrack, P. & Pigeon, M. (1989) *J. Immunol.* **142,** 2736–2742.
25. Lapeyre, C., Kaveri, S. V., Janin, F. & Strosberg, A. D. (1987) *Mol. Immunol.* **24,** 1243–1254.
26. Samelson, L. E., Germain, R. N. & Schwartz, R. H. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 6972–6976.
27. Gascoigne, N. R. J., Goodnow, C. C., Dudzik, K. I., Oi, V. T. & Davis, M. M. (1987) *Proc. Natl. Acad. Sci. USA* **84,** 2936–2940.
28. Quaranta, V., Walker, L. E., Pellegrino, M. A. & Ferrone, S. (1980) *J. Immunol.* **125,** 1421–1425.
29. Barnstable, C. J., Bodmer, W. F., Brown, G., Galfre, G., Milstein, C., Williams, A. F. & Zeigler, A. (1978) *Cell* **14,** 9–20.
30. Calman, A. F. & Peterlin, B. M. (1987) *J. Immunol.* **139,** 2489–2495.
31. de Preval, C. & Mach, B. (1983) *Immunogenetics* **17,** 133–140.
32. Chien, Y., Gascoigne, N. R. J., Kavaler, J., Lee, N. E. & Davis, M. M. (1984) *Nature (London)* **309,** 322–326.
33. Doyle, C. & Strominger, J. L. (1987) *Nature (London)* **330,** 256–259.
34. Norment, A. M., Salter, R. D., Parham, P., Engelhard, V. H. & Littman, D. R. (1988) *Nature (London)* **336,** 79–81.
35. Herrmann, T., Maryanski, J. L., Romero, P., Fleischer, B. & MacDonald, H. R. (1990) *J. Immunol.* **144,** 1181–1186.
36. Kanagawa, O. & Ahlem, C. (1989) *Int. Immunol.* **1,** 565–569.