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Immunology

Antagonism of superantigen-stimulated helper T-cell clones and hybridomas by altered peptide ligand

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ABSTRACT **T-cell activation by an immunogenic peptide can be antagonized by nonstimulatory analogs of that peptide. We investigated this T-cell receptor antagonism by using staphylococcal enterotoxin superantigen to stimulate hemoglobin-specific helper T (T_h) cells because its activation pathway may differ from that of conventional antigen. Interestingly, superantigen activation of these T_h cells was antagonized by hemoglobin peptide analogs even though agonist (superantigen) and antagonist (analog peptide) bind at different sites on the major histocompatibility complex-encoded molecule and the T-cell receptor. The antagonism appeared to be a fundamental block in T-cell activation, as phosphoinositol generation, cytokine production, and proliferation were reduced in T_{h1} clones, and, similarly, proliferative and cytokine responses were inhibited in T_{h2} cells. Even T-cell hybridoma activation (cytokine production and apoptosis) was inhibited by peptide antagonists. Furthermore, analog peptides that functioned as partial agonists for these T_h cells also antagonized superantigen-induced proliferation and thus were a subset of the peptide antagonists. In summary, our results demonstrate that analogs of immunogenic peptide are potent antagonists for T_h cell responses induced by superantigen as well as immunogenic peptide.**

For $CD4^+$ T cells, stimulatory ligands for the T-cell receptor (TCR) can be produced by the combination of processed peptides or staphylococcal enterotoxin (SE) superantigens bound to major histocompatibility complex (MHC) class II molecules (1, 2). TCR interaction with either ligand can activate a cascade of cellular events that may lead to cytokine production, cell division, or cell death (3–6). Peptide and superantigen activate these T-cell responses by forming a trimolecular receptor–ligand complex with the TCR and MHC class II molecule, but the mechanism of activation may differ, as peptide and superantigen interact at spatially distinct regions on the MHC class II molecule and the TCR (7–11). Consistent with this possibility is evidence that the two ligands induce different intracellular signals (12, 13).

In receptor–ligand interactions, alteration of the ligand may change the response initiated through the receptor. The immunogenic peptide ligand for the TCR can be modified by introducing single amino acid substitutions to create analog peptides. These substituted peptides can subsequently be used to identify the amino acids in the peptide that are critical for T-cell responses. We have defined the term "altered peptide ligand" (APL) as analog peptides which contain these single amino acid changes (14). In our murine hemoglobin [Hbβ^d-(64–76) peptide] model, APLs were identified for a panel of helper T (T_h) cell clones and T-cell hybridomas (15). For the T_h clones, some APLs stimulated the T_h cells to produce cytokine, lyse target cells, or induce T-cell anergy in the absence of T-cell proliferation (16–18). The incomplete nature of the T-cell response (absence of proliferation) suggested that these APLs were partial agonists for the TCR. In a similar fashion, peptide antagonists for the TCR have been reported by Sette and coworkers (19–22) for human T_h clones stimulated by an immunogenic peptide. For those studies, the presence of APL antagonists together with an immunogenic peptide blocked the induction of T-cell responses. Peptide antagonists for the TCR have subsequently been described for murine $CD8^+$ clones and a $CD4^+$ T_{h1} clone (23, 24).

In the studies reported here, we have attempted to extend our findings with APLs to identify antagonists for T_h cell responses and to ascertain the relationship between partial agonists and antagonists. We found that APLs previously identified as partial agonists effectively antagonized proliferative responses of T_h clones and that APLs could antagonize T_{h1} responses as well as T_{h2} and T-cell hybridoma responses. In addition, APLs blocked T-cell responses when superantigens were used as an alternative stimulatory ligand for the TCR. These experiments continue our investigations into the specificity of interaction between the TCR and peptide ligand.

MATERIALS AND METHODS

Reagents. Female CBA/J and B10.BR mice (5–10 weeks of age) were purchased from the National Cancer Institute or The Jackson Laboratory. The Hbβ^d-(64–76) peptide and the analog APLs were synthesized on a DuPont RaMPS apparatus and purified by reverse-phase HPLC, and amino acid content was confirmed by analysis on a Beckman 6300 amino acid analyzer. Staphylococcal enterotoxin A (SEA) and anti-CD28 monoclonal antibody (mAb) 37.51 were purchased from Toxin Technology (Sarasota, FL) and PharMingen, respectively. Murine recombinant interleukin 1β (IL-1β) was a gift of David D. Chaplin (Washington University).

Cell Lines. We previously described the generation and characterization of a panel of Hb-reactive T_h clones, including the T_{h2} clone 2.102 and the T_{h1} clones PL.17 and Hb#2 (15). These T_h cells all use the $V_{\beta 1}$ chain for their TCR and are activated by the SEA superantigen (B. Hsu, personal communication). The G2 T-cell hybridomas were made as described by using the T_{h2} 2.102 clone and the $\alpha^-\beta^-$BW5147 fusion partner (17, 25). The CH27 B-cell lymphoma which was used as the antigen-presenting cell (APC) expresses H-2^k, B7, and ICAM-1 (ref. 26 and B.D.E., unpublished data).

TCR Antagonism. TCR antagonism was assayed by T_h proliferation or cytokine production using a modification of a published method (19, 20). The APCs (CH27) were mitomycin C-treated (50 μg/ml) and prepulsed with Hbβ^d-(64–76) or superantigen as stimulus for 2 hr, at which time the unbound stimulus was removed. The proliferative responses of the T_h clones were assayed in 96-well flat-bottomed plates

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Abbreviations: APC, antigen-presenting cell; APL, altered peptide ligand; IL, interleukin; mAb, monoclonal antibody; MHC, major histocompatibility complex; SEA, staphylococcal enterotoxin A; TCR, T-cell receptor; T_h, helper T.

in 200 μl of RPMI 1640 medium containing the clones (3–10 × 10^4 cells per well), CH27 cells, and analog Hb peptides (0–100 μM). The presentation of immunogenic peptide or superantigen by the APCs activated the T_h cells, and the antagonist peptides inhibited this level of T_h stimulation. For T_h clone proliferation assays, cells were incubated with [*methyl*-^{3}H]thymidine (0.4 μCi per well; 1 μCi = 37 kBq) after 48 hr of culture and harvested the next day.

T-Cell Lymphokine Responses. In some experiments, lymphokine responses (IL-2 or IL-3) of the T_h cells were quantitated by bioassay on 24-hr supernatants by using the IL-2-dependent cell line CTLL-2 and the IL-3-dependent cell line GG1.12 (15, 17). The indicator cells were incubated with test supernatants for 48 hr. [^{3}H]Thymidine was included during the final 20 hr.

Inositol Phosphate Metabolism. Inositol phosphates were assayed as described (18). Briefly, T_{h1} Hb#2 cells (1–2 × 10^7 cells per ml) were loaded with *myo*-inositol (20–50 μCi/ml; Amersham) overnight in inositol-free medium. Superantigen (SEA) was incubated with the APCs for 2 hr before initiation of the assay. After a 90-min incubation with APCs (7–10 × 10^5 CH27 cells per well), inositol phosphates were extracted from the T_h cells (7–10 × 10^5 cells per well) by ion-exchange chromatography, with the total inositol phosphates (radiolabel) determined by liquid scintillation counting.

RESULTS

APLs Block the Superantigen Stimulation of T_{h1} and T_{h2} Clones. We previously used APLs with similar affinities for the MHC molecule to identify the amino acids of an immunogenic Hb peptide [Hbβ^d-(64–76)] that contact the TCR for a panel of T_{h1} and T_{h2} clones (15). To assay antagonism, these APLs were simultaneously presented by APCs that had been preloaded with a superantigen stimulus. For the T_{h2} clone 2.102, amino acids at positions 69, 72, 73, and 74 are critical for clonal proliferation (15, 16). While the Gln^{72} peptide was unable to block superantigen-induced proliferation (Fig. 1*A*), APLs containing substitutions at position 69, 73, or 74 prevented the proliferation of the T_{h2} clone (Fig. 1*A*). As these peptides blocked the T_{h2} proliferative response to the superantigen stimulus, they were T-cell antagonists. In a similar fashion, the superantigen stimulation of the T_{h1} clone PL.17 was also affected by some APLs (Fig. 1*B*). The Ser^{70} and Asn^{73} peptides prevented the proliferative response against SEA, whereas the Gln^{72} peptide had no effect. IL-3 production was inhibited by the APLs in both the T_{h1} and T_{h2} clones (data not shown). Thus, APLs could antagonize superantigen-activated T_{h1} and T_{h2} clones.

APLs Block the Immunogenic Peptide Stimulation of T_{h1} and T_{h2} Clones. The APLs were also used to antagonize T_h cell responses stimulated by an immunogenic peptide. APLs with changes at amino acids 69, 73, and 74 were antagonists of proliferation in the T_{h2} 2.102 cells (Fig. 2*A* and data not shown), but the Gln^{72} peptide and a control I-E^k-binding peptide [moth cytochrome-*c*-(89–103); Fig. 2*A*] did not affect the response. Similarly, for the T_{h1} clone PL.17, APLs containing substitutions at residues 70 and 73 lowered proliferation in response to the immunogenic peptide (Fig. 2*B*) whereas the Gln^{72} peptide again did not change the response. Interestingly, the Ser^{70} peptide by itself on live APCs induced the PL.17 clone into a state of anergy, defining it as an APL partial agonist (18), but in the presence of an immunogenic peptide this Ser^{70} APL antagonized the proliferative response. The responses of the T_h clones to the various APLs are summarized in Fig. 3. These experiments confirmed that APLs can antagonize immunogenic peptide-stimulated T_{h1} responses and extended the findings to also include a T_{h2} clone.

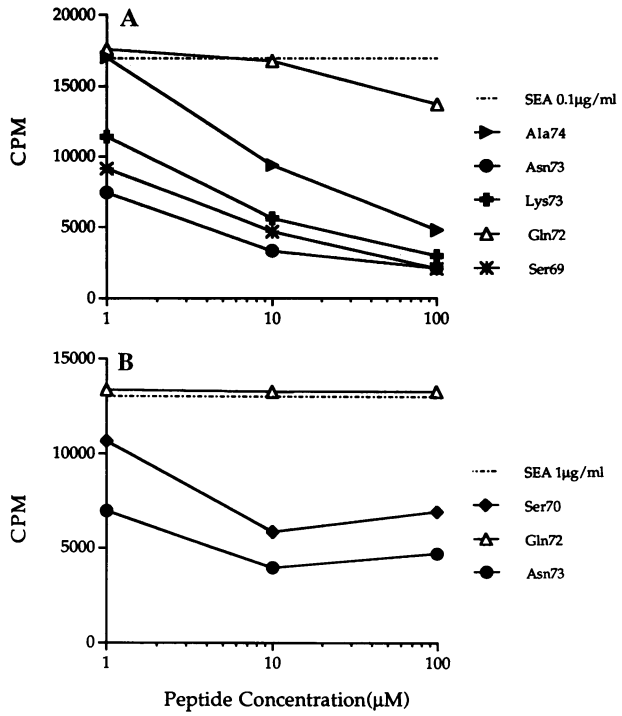

FIG. 1. APLs inhibit the proliferative response of T_{h1} and T_{h2} clones stimulated with a superantigen. (*A*) T_{h2} 2.102 cells (5 × 10^4) were cultured for 3 days with APCs (CH27) prepulsed with SEA (0.1 μg/ml). The cultures contained the indicated amount of competitor peptide or medium alone. The T-cell proliferative response induced by the APCs plus SEA is depicted as a broken line. Analog Hb peptides are referred to by their three-letter code (Gln^{72} signifies that glutamine was substituted at position 72). Means of duplicate cultures are shown with SD < 15%. (*B*) T_{h1} PL.17 cells were cultured for 3 days with APCs prepulsed with SEA (1 μg/ml). T-cell proliferative response induced by SEA at 1 μg/ml is depicted as a broken line. The data represent the means of triplicate cultures with SD < 15% of the mean.

APLs Block the Superantigen Activation of Hb-Reactive T-Cell Hybridomas. Since APLs could antagonize the T_h clones, T-cell hybridomas produced from the clones may also be susceptible to antagonism. Hybridomas differ from T_h clones in that most hybridoma cells do not require costimulatory signals for maximal responses. For both the G2 T-cell hybridoma and its parent T_{h2} clone, 2.102, amino acids at positions 69, 72, 73, and 74 are critical for T-cell activation. APLs which antagonized the T-cell hybridoma IL-2 response (Ser^{69}, Asn^{73}, and Ala^{74}; Fig. 4*A*) also antagonized the parent T_{h2} clone response (proliferation, Fig. 1*A*). Antagonism was observed in the hybridoma assays using mitomycin C-treated or chemically fixed APCs (Fig. 4*A* and data not shown).

Activation of T-cell hybridomas also induces apoptosis in addition to cytokine production, and this apoptosis appears similar to the programmed cell death observed in thymocytes (27). The induction of apoptosis by a TCR stimuli (peptide or superantigen) leads to decreased T-cell hybridoma proliferation (27, 28). Therefore, apoptosis in T-cell hybridomas can be measured as diminished proliferation. The Asn^{73} and Ser^{69} APLs blocked the hybridoma apoptotic response induced by superantigen, whereas the control Gln^{72} peptide did not (Fig. 4*B*). Thus, APLs inhibited superantigen activation of T-cell hybridomas as measured by lymphokine production or apoptosis.

In the published reports of antagonism and the experiments described above, immunogenic peptide was added to the APCs and excess peptide was removed prior to the addition of the APLs to minimize competition for the MHC molecule.

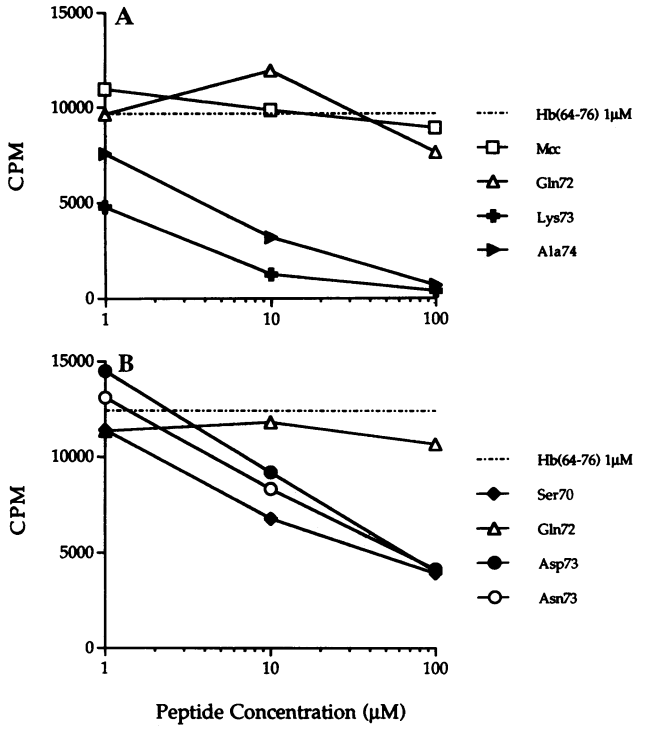

FIG. 2. APLs inhibit the proliferative response of T_{h1} and T_{h2} clones stimulated with the immunogenic peptide. (*A*) T_{h2} 2.102 cells (5×10^4) were cultured for 3 days with APCs (CH27) prepulsed with 1 μM Hbβ^d-(64–76). Details of the experiment are the same as for Fig. 1. Moth cytochrome-*c* peptide (Mcc) was included as a control for binding to the MHC I-E^k molecule. The mean of duplicate cultures is shown. (*B*) T_{h1} PL.17 cells (3×10^4) were cultured for 3 days with APCs (CH27) prepulsed with 1 μM Hbβ^d-(64–76). T-cell proliferative response induced by the APCs and 1 μM Hb peptide is depicted as a broken line. The data represent the means of triplicate cultures.

The absence of competition between superantigen and peptide for the MHC molecule allowed the simultaneous addition of both to the APCs. Under these conditions, the Asn^{73} peptide (100 μM) blocked (>90%) T-cell hybridoma activation in response to SEA, while the response was unaffected by the Gln^{72} peptide. The lack of competition between superantigen and peptide for the MHC molecule indicated that the APL antagonists were inhibiting T-cell activation through the TCR, as has also been recently described for human T_h cells (22).

Exogenous Costimulatory Signals Do Not Overcome the Peptide Antagonism. Costimulatory signals are required for optimal T-cell clonal proliferation and lymphokine responses

Peptide		Th1 PL.17 Peptide +APC	Th1 PL.17 Antagonism Assay	Th2 2.102 Peptide +APC	Th2 2.102 Antagonism Assay
Hb(64-76)	GKKVITAFNEGLK	Agonist	ø	Agonist	ø
Ser69	-----S-------	Agonist	ø	No Response	+
Ser70	------S------	Partial Agonist	+	Agonist	ø
Gln72	--------Q----	No Response	–	No Response	–
Asp73	---------D---	Partial Agonist	+	Partial Agonist	+
Asn73	---------N---	No Response	+	No Response	+
Lys73	---------K---	No Response	+	No Response	+
Ala74	----------A--	Agonist	ø	No Response	+

FIG. 3. Peptides are shown in the single-letter amino acid code. APL classifications are based on the support of T_h clonal proliferation. Agonists support proliferative responses, whereas partial agonists do not support proliferation but do functionally activate some T_h responses (16, 18). The no-response APLs failed to activate the T_h cells. Antagonism assay was performed as described with SEA- and Hbβ^d-(64–76)-stimulated T_h clones. Agonist peptides indicated by "ø" by definition could not be used in the antagonism assay.

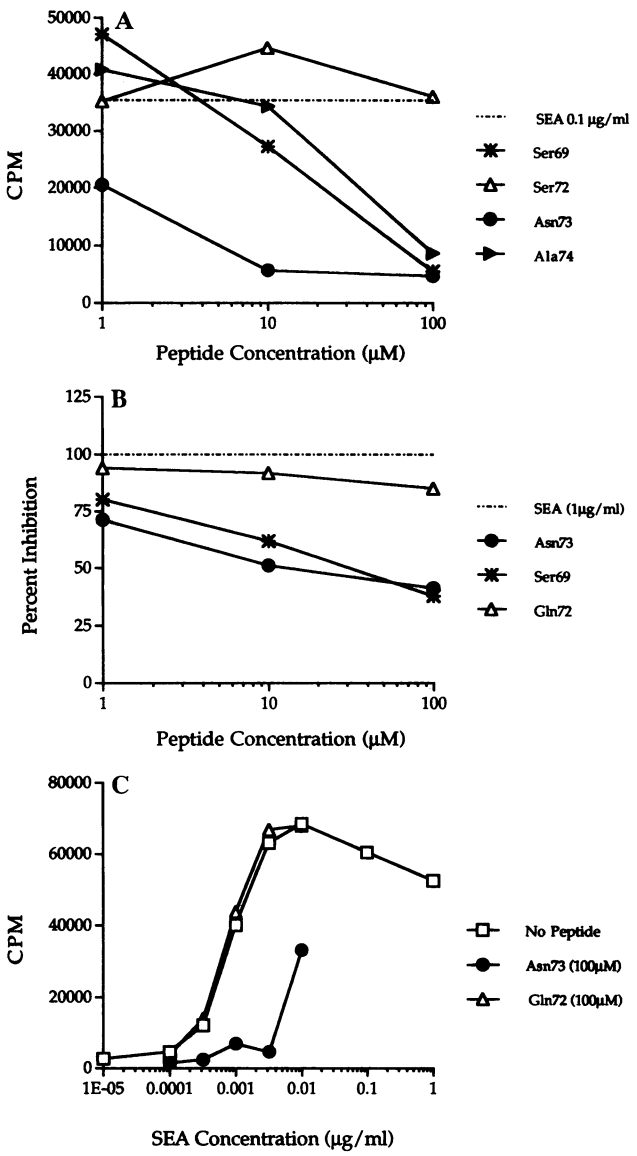

FIG. 4. APLs inhibit the responses of the Hb-reactive T-cell hybridoma G2. (*A*) IL-2 response was assayed by culturing G2 cells (10^5) for 1 day with APCs (CH27) prepulsed with SEA (0.1 μg/ml). (*B*) Apoptotic response was assayed by culturing G2 cells (2×10^4) for 1 day with APCs prepulsed with SEA (1 μg/ml). Data are shown as percent inhibition of apoptosis induction as measured by hybridoma proliferation. The proliferative response of the hybridoma cells to this concentration of SEA (9000 cpm) was standardized as 100% inhibition of proliferation (broken line). The Asn^{73} and Ser^{69} peptides increased the hybridoma proliferation (22,000 and 24,000 cpm, respectively), indicating that apoptosis had been inhibited. Background proliferation of the mitomycin C-treated APCs alone was 5300 cpm. (*C*) The Asn^{73} APL inhibits T-cell hybridoma IL-2 response to SEA when added simultaneously to culture. G2 cells (10^5) were cultured for 1 day with APCs and the indicated concentration of SEA. The Gln^{72} and Asn^{73} APLs were added at 100 μM. Both the superantigen and peptides were simultaneously present throughout the culture period.

(29), and they have been suggested to be important factors in TCR antagonism (24). The role of costimulation in APL antagonism was examined by adding recombinant IL-1β or an anti-CD28 mAb as exogenous costimulus (30, 31). APL antagonists were effective at inhibiting the T_{h2} proliferative response to SEA in the presence or absence of exogenous costimulation (IL-1β or anti-CD28) (Fig. 5*A*). Interestingly, both IL-1β and the anti-CD28 mAb increased the total proliferative response of the T_{h2} clone in the assay, which was

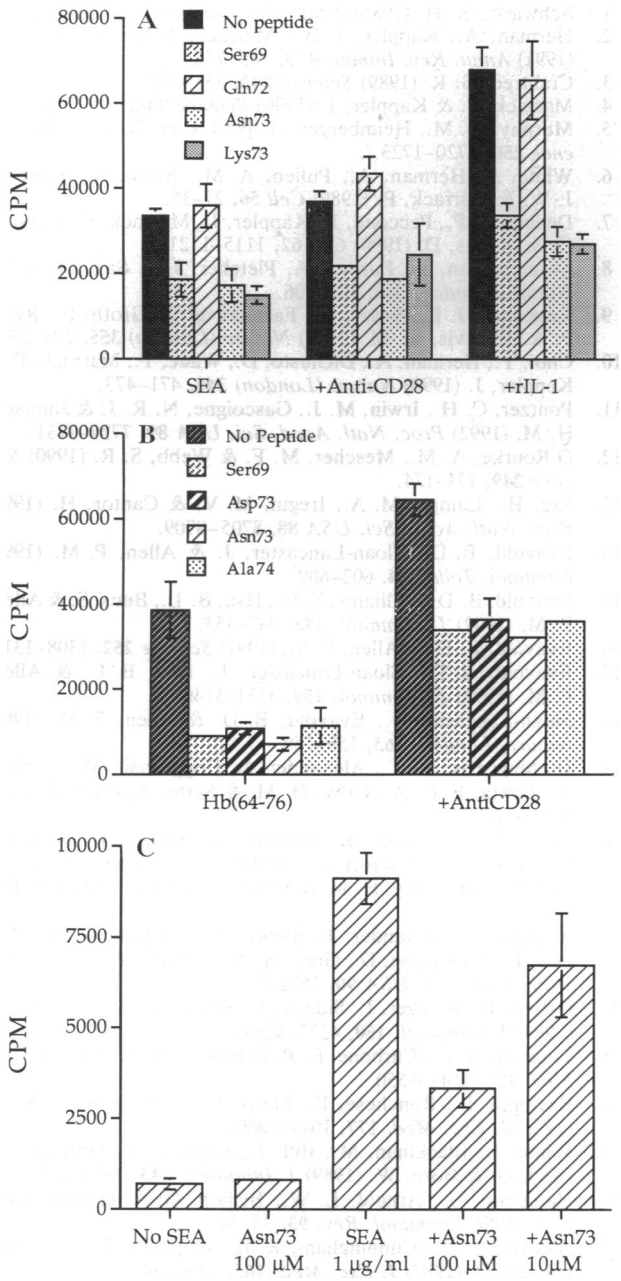

FIG. 5. APL antagonism is not changed by exogenous costimulatory signals in T_h clones and it does not induce phosphatidylinositol turnover in T_{h1} clones. (*A*) T_{h2} 2.102 cells (5×10^4) were cultured for 3 days with APCs prepulsed with SEA (1 μg/ml). Recombinant (r) IL-1β (100 pg/ml) or anti-CD28 mAb (1 μg/ml, then crosslinked with goat anti-hamster antibody) was added to the T_{h2}/APC cultures. Only the 100 μM concentration of antagonist peptides are shown. (*B*) T_{h1} Hb#2 cells (3×10^4) were cultured for 3 days with APCs prepulsed with 10 μM $Hb\beta^d$-(64–76). Anti-CD28 mAb (1 μg/ml, then crosslinked with goat anti-hamster antibody) was added to the T_{h1}/APC cultures. Only the values from the 100 μM concentration of antagonist peptides are shown. (*C*) APLs inhibit the SEA generation of free inositol phosphates by T_{h1} clone Hb#2 clone. T_{h1} cells were labeled with *myo*-[^{3}H]inositol before use and added (8.5×10^5 cells per well) to APCs (CH27), prepulsed for 2 hr with SEA (1 μg/ml). After 90 min, free inositol phosphates were quantitated. Means of duplicate cultures are shown.

probably a consequence of using a submaximal concentration of SEA to stimulate the T cells. Costimulation also failed to change the antagonism of the T_{h1} clone Hb#2 stimulated with immunogenic peptide. For this clone, APLs with substitutions positions 69, 73, and 74 inhibited the proliferative response (Fig. 5*B*). Interestingly, the Asp^{73} APL was an antagonist when used with immunogenic peptide (Fig. 5*B*), but this peptide was a partial agonist for this T_{h1} clone when presented alone by APCs (17). As was observed with the T_{h2} clone, exogenous costimulation increased the magnitude of the T_{h1} proliferative response with no observable change in the antagonism.

APLs Block the Superantigen Stimulation of Phosphatidylinositol Metabolism in T_{h1} Clones. As the addition of exogenous costimuli did not alter the APL antagonism, these peptides could be affecting an earlier step in the TCR signaling pathway. Total phosphatidylinositol metabolites were assayed in T_{h1} clones Hb#2 and PL.17 to investigate this possibility. Superantigen stimulated the T_{h1} clone Hb#2 to generate phosphatidylinositol metabolites, whereas Asn^{73} APL alone caused no detectable signal (Fig. 5*C*). Superantigen and Asn^{73} APL together, however, resulted in decreased production of inositol phosphates, similar to the antagonism reported for human T_h clones and immunogenic peptide (21). Although superantigen and conventional antigen can differ in the signals transduced via the TCR, typical T-cell signals (Ca^{2+} flux, phosphatidylinositol metabolism and phospholipase C-γ1 phosphorylation) have been observed following superantigen activation of some T cells (12, 13, 32–34). At least for the induction of phosphatidylinositol turnover, superantigen and conventional antigen appeared to activate similar pathways in our T_{h1} clones.

DISCUSSION

Agonist, partial agonist, and antagonist ligands are identifiable for any receptor. This allows a given receptor to variably respond with gradations of signals and biological responses. The TCR should similarly be capable of variable responses to agonist, partial agonist, and antagonists. Our previous experiments with APLs have identified partial agonists for T_h cells (16–18), and here we have identified APL antagonists. TCR antagonism has been reported to reduce most aspects of T-cell activation, including proliferation, phosphatidylinositol turnover, cytolysis, and Ca^{2+} signaling (19–24). Our data are compatible with many of these findings and extend them to include antagonism of a T_{h2} clone as well as T-cell hybridoma responses (Figs. 1*A* and 4). For the T_h clones, APL antagonists blocked the cytokine and proliferative responses, which were not restored by the presence of exogenous costimulatory signals (Fig. 5 *A* and *B*). The lack of an effect with costimulation was consistent with the findings that T-cell hybridoma responses were susceptible to APL antagonists (Fig. 4). Previously, another group was unable to demonstrate antagonism in a T-cell hybridoma (24). While we have not identified the reason for this discrepancy between the hybridoma data, it could be related to the mechanism of T-cell activation (alloantigen vs. superantigen/immunogenic peptide), the type of APC (transfected L cell vs. B-cell lymphoma), or an inherent variability among T-cell hybridomas. It is unlikely that the parental T_h clone used to generate the hybridomas (T_{h1} vs. T_{h2}) affects the susceptibility of hybridoma cells to antagonism, because responses were inhibited in cells made from T_{h1} or T_{h2} clones (Fig. 4 and B.D.E., unpublished data).

In addition, we found that APL antagonists were effective at inhibiting superantigen activation of T_h cell responses (Fig. 1). This was important because it showed that the antagonists were specific for the receptor and not for the stimulatory ligand used to activate the T cells. Superantigen was a valuable ligand for T-cell responses because it interacted with both the MHC molecule and the TCR at distinct sites from the peptide ligand (7, 8, 10, 11). The absence of competition for MHC molecules definitively showed that APLs inhibited

T-cell responses through the TCR (Fig. 4*C*), and suggested that the antagonism operated via a signaling event.

While a relationship between TCR signals and antagonism has been proposed (21, 23, 24), the APLs themselves did not generate traditional T-cell signals (Fig. 5*C* and refs. 19, 21, 23, and 24). The APL partial agonists were interesting because they tolerized T_{h1} clones (Ser^{70}), supported T_{h1} cytolysis (Asp^{73}), and induced B-cell help (Asp^{73}) without stimulating T-cell proliferation when presented alone by APCs (16–18). Their inability to support clonal proliferation suggested that they differed qualitatively from the immunogenic peptide. When these partial agonists and stimulatory ligand were presented together by APCs, they antagonized T_h responses (Figs. 2, 3, and 5). As tolerance, cytolysis, and help required active T-cell processes, these APLs initiated some form of T-cell signal; however, we cannot argue that all antagonists transmit a signal through the TCR (Figs. 2, 3, and 5).

Other groups have identified TCR antagonists with mixed partial agonist/antagonist or agonist/antagonist properties that may be similar to our partial agonist peptides (20, 23, 24), but the antagonists used in those systems were not tested for the functional T-cell responses that we have used to identify APL partial agonists. The APL partial agonists are incapable of activating T_h clone proliferation but do activate functional responses as well as weakly activate T-cell hybridomas (17). Based on these observations, we would expect the antagonist for the system of Racioppi *et al.* (24) to be a partial agonist, since it displays a similar phenotype.

The variation of TCR interaction seen with APLs led to their suggested role in the selection of thymocytes (17, 23, 24). This interaction between TCR and APL could be similar to that proposed in the affinity model of thymocyte maturation (35). If the apoptotic response of T-cell hybridomas is comparable to programmed cell death in thymocytes (28), then our hybridoma experiments are a clear demonstration that a nonproliferative APL can antagonize apoptosis. The hybridoma experiment may be more applicable to negative selection, since the superantigen stimulus was prevented from inducing apoptosis, but positive selection may also be affected. Again, a role for endogenous peptides in positive and negative selection supports APLs delivering a signal to the TCR.

The ability of peptide antagonists to modulate superantigen stimulation in T_h cells may also have important therapeutic applications. The potent nature of the antagonism (Fig. 4*C*) suggests that it may be possible to block superantigen activation and deletion of T_h cells *in vivo*. Superantigen activation of T_h responses is critical for infection by a multitude of bacterial and viral organisms (4, 36). Our data suggest that T_h responses to superantigen could be potentially treated with a mixture of peptides which would thereby prevent infection. A combination of peptides would be necessary to block the polyclonal nature of the T-cell response to superantigen. This modality is based upon the observations that APL antagonists can prevent the TCR response to superantigen and is derived from the idea that the TCR responds to ligand with more than a simple on/off signal with all stimuli leading to clonal proliferation.

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1. Schwartz, R. H. (1985) *Annu. Rev. Immunol.* **3**, 237–261.
2. Herman, A., Kappler, J. W., Marrack, P. & Pullen, A. M. (1991) *Annu. Rev. Immunol.* **9**, 745–772.
3. Crabtree, G. R. (1989) *Science* **243**, 355–361.
4. Marrack, P. & Kappler, J. (1990) *Science* **248**, 705–711.
5. Murphy, K. M., Heimberger, A. B. & Loh, D. Y. (1990) *Science* **250**, 1720–1723.
6. White, J., Herman, A., Pullen, A. M., Kubo, R., Kappler, J. W. & Marrack, P. (1989) *Cell* **56**, 27–35.
7. Dellabona, P., Peccoud, J., Kappler, J., Marrack, P., Benoist, C. & Mathis, D. (1990) *Cell* **62**, 1115–1121.
8. Swaminathan, S., Furey, W., Pletcher, J. & Sax, M. (1992) *Nature (London)* **359**, 801–806.
9. Jorgensen, J. L., Esser, U., Fazekas de St. Groth, B., Reay, P. A. & Davis, M. M. (1992) *Nature (London)* **355**, 224–230.
10. Choi, Y., Herman, A., DiGiusto, D., Wade, T., Marrack, P. & Kappler, J. (1990) *Nature (London)* **346**, 471–473.
11. Pontzer, C. H., Irwin, M. J., Gascoigne, N. R. J. & Johnson, H. M. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7727–7731.
12. O'Rourke, A. M., Mescher, M. F. & Webb, S. R. (1990) *Science* **249**, 171–174.
13. Liu, H., Lampe, M. A., Iregui, M. V. & Cantor, H. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 8705–8709.
14. Evavold, B. D., Sloan-Lancaster, J. & Allen, P. M. (1993) *Immunol. Today* **14**, 602–609.
15. Evavold, B. D., Williams, S. G., Hsu, B. L., Buus, S. & Allen, P. M. (1992) *J. Immunol.* **148**, 347–353.
16. Evavold, B. D. & Allen, P. M. (1991) *Science* **252**, 1308–1310.
17. Evavold, B. D., Sloan-Lancaster, J., Hsu, B. L. & Allen, P. M. (1993) *J. Immunol.* **150**, 3131–3140.
18. Sloan-Lancaster, J., Evavold, B. D. & Allen, P. M. (1993) *Nature (London)* **363**, 156–159.
19. De Magistris, M. T., Alexander, J., Coggeshall, M., Altman, A., Gaeta, F. C. A., Grey, H. M. & Sette, A. (1992) *Cell* **68**, 625–634.
20. Alexander, J., Snoke, K., Ruppert, J., Sidney, J., Wall, M., Southwood, S., Oseroff, C., Arrhenius, T., Gaeta, F. C. A., Colón, S. M., Grey, H. M. & Sette, A. (1993) *J. Immunol.* **150**, 1–7.
21. Ruppert, J., Alexander, J., Snoke, K., Coggeshall, M., Herbert, E., McKenzie, D., Grey, H. M. & Sette, A. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 2671–2675.
22. Ostrov, D., Krieger, J., Sidney, J., Sette, A. & Concannon, P. (1993) *J. Immunol.* **150**, 4277–4283.
23. Jameson, S. C., Carbone, F. R. & Bevan, M. J. (1993) *J. Exp. Med.* **177**, 1541–1550.
24. Racioppi, L., Ronchese, F., Matis, L. A. & Germain, R. N. (1993) *J. Exp. Med.* **177**, 1047–1060.
25. White, J., Blackman, M., Bill, J., Kappler, J., Marrack, P., Gold, D. & Born, W. (1989) *J. Immunol.* **143**, 1822–1825.
26. Haughton, G., Arnold, L. W., Bishop, G. A. & Mercolino, T. J. (1986) *Immunol. Rev.* **93**, 35–51.
27. Ashwell, J. D., Cunningham, R. E., Noguchi, P. D. & Hernandez, D. (1987) *J. Exp. Med.* **165**, 173–194.
28. Mercep, M., Weissman, A. M., Frank, S. J., Klausner, R. D. & Ashwell, J. D. (1989) *Science* **246**, 1162–1165.
29. Schwartz, R. H. (1990) *Science* **248**, 1349–1356.
30. Rojo, J. R., Kerner, J. D. & Janeway, C. A., Jr. (1989) *Eur. J. Immunol.* **19**, 2061–2067.
31. Harding, F. A., McArthur, J. G., Gross, J. A., Raulet, D. H. & Allison, J. P. (1992) *Nature (London)* **356**, 607–609.
32. Fraser, J. D., Newton, M. E. & Weiss, A. (1992) *J. Exp. Med.* **175**, 1131–1134.
33. Chatila, T., Wood, N., Parsonnet, J. & Geha, R. S. (1988) *J. Immunol.* **140**, 1250–1255.
34. Fleischer, B. & Schrezenmeier, H. (1988) *J. Exp. Med.* **167**, 1697–1707.
35. von Boehmer, H., Teh, H. S. & Kisielow, P. (1989) *Immunol. Today* **10**, 57–61.
36. Held, W., Waanders, G. A., Shakhov, A. N., Scarpellino, L., Acha-Orbea, H. & MacDonald, H. R. (1993) *Cell* **74**, 529–540.