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Immunology

Interleukin 2 production, not the pattern of early T-cell antigen receptor-dependent tyrosine phosphorylation, controls anergy induction by both agonists and partial agonists

(T cell/antigen/receptor/major histocompatibility complex class II)

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ABSTRACT **Full activation of T cells requires signaling through the T-cell antigen receptor (TCR) and additional surface molecules interacting with ligands on the antigen-presenting cell. TCR recognition of agonist ligands in the absence of accessory signals frequently results in the induction of a state of unresponsiveness termed anergy. However, even in the presence of costimulation, anergy can be induced by TCR partial agonists. The unique pattern of early receptor-induced tyrosine phosphorylation events induced by partial agonists has led to the hypothesis that altered TCR signaling is directly responsible for the development of anergy. Here we show that anergy induction is neither correlated with nor irreversibly determined by the pattern of early TCR-induced phosphorylation. Rather, it appears to result from the absence of downstream events related to interleukin 2 receptor occupancy and/or cell division. This implies that the anergic state can be manipulated independently of the precise pattern of early biochemical changes following TCR occupancy, a finding with implications for understanding the induction of self-tolerance and the use of partial agonist ligands in the treatment of autoimmune diseases.**

T-cell tolerance to self-components is achieved by intrathymic deletion (1) and additional peripheral mechanisms, including apoptotic death (2–4), immunoregulation (5), and clonal anergy (6, 7). This latter state of unresponsiveness to T-cell antigen receptor (TCR) stimulation may be especially important in achieving tolerance to tissue-specific antigens on non-hematopoietic cells. Two signal models of T-cell activation (8, 9) have proposed that TCR occupancy by a peptide/major histocompatibility complex (MHC) molecule ligand ("agonist") triggers an intracellular event ("signal 1"), which, without accessory or costimulatory signals ("signal 2"), leads to the anergic state (10–13). These conditions of TCR engagement without costimulation are believed to apply to most somatic cells lacking professional antigen-presenting cell (APC) function, accounting for tolerance induction to their displayed peptide/MHC molecule ligands.

Recently, Sloan-Lancaster *et al.* (14, 15) have shown that variant TCR ligands lacking the ability to stimulate some or all T-cell effector activities ("partial agonists") can also induce anergy, even when presented by costimulatory APCs. This induction of unresponsiveness was reported to occur without stimulation of changes in intracellular Ca^{2+} concentrations or induction of inositol phosphate hydrolysis, despite the ability of cyclosporin A to prevent anergy. More recently, both Sloan-Lancaster *et al.* (15) and Madrenas *et al.* (16) have described a novel pattern of early (2–10 min) TCR-associated tyrosine phosphorylation after exposure of T cells to variant peptide/MHC molecule combinations. Partial agonist stimulation of mouse T cells results in the following: (*i*) a predominance of the p21 phosphorylated form of the TCR ζ chain, rather than the similar levels of both the p21 and p23 forms seen with agonist; (*ii*) little or no CD3ε tyrosine phosphorylation; and (*iii*) association of the *syk* family kinase ZAP-70 with the phosphorylated ζ chains without the stable enzymatic activation of this key molecule. Based on this distinctive pattern of TCR-dependent phosphorylation associated with presentation of partial agonists, it has been suggested that disturbed intracellular signaling directly induces the anergic state (15).

This hypothesis of a special role for altered signaling in anergy induction is distinct from previous results in anergy models using agonist presentation in the absence of CD28-dependent costimulation, which show that either interleukin 2 (IL-2; refs. 17 and 18) or antibody-induced signaling through the common γ chain of the IL-2, -4, and -7 receptors (19) can prevent or reverse anergy. The former studies concluded that it was the absence of IL-2-mediated signaling and/or proliferation that led to the anergic state, not a change in TCR signal transduction in the presence and absence of costimulation. A unique role for variant TCR signaling in inducing unresponsiveness also seems inconsistent with limited biochemical studies of signal transduction during anergy induction in the absence of CD28-related costimulation (11, 20, 21). These previous experiments did not specifically look at ZAP-70 or compare distinct ζ-chain phosphorylation states, however, and were conducted solely with agonist ligands. This left open the possibility that differences in early steps in the signaling cascade induced by typical agonist ligands on live, costimulatory cells as compared with fixed antigen presenting cells, soluble concanavalin A, or plate-bound anti-CD3 antibody might have played a crucial role in inducing the unresponsive state, in accord with the hypothesis based on the properties of partial agonist ligands. In addition, no data are available concerning whether IL-2 might play a different role in the two models of anergy.

Distinguishing between these possibilities has important implications for our overall understanding of TCR signaling, as well as with respect to the potential use of variant ligands in particular, and anergy induction in general, as a strategy for treatment of autoimmune diseases. We have therefore reexamined the TCR-associated tyrosine phosphorylation events accompanying agonist or partial agonist stimulation under

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Abbreviations: TCR, T-cell antigen receptor; MHC, major histocompatibility complex; APC, antigen-presenting cell; IL-2, interleukin 2; PCC, pigeon cytochrome *c*; MCC, moth cytochrome *c*.

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conditions leading to anergy, as well as the role of IL-2 in regulating the unresponsive state. Our experiments support the view that the extent of IL-2 production, and not the specific pattern of early TCR-associated tyrosine phosphorylation events, is the key factor determining whether anergy ensues in both situations. These data indicate that paracrine cytokine secretion may play an important role in regulating the effector functions of autoreactive T cells and suggest the possibility that signaling in response to variant ligands, which is not readily revealed by the standard analysis of tyrosine phosphorylated proteins, may occur and contribute to a number of the functional properties of these ligands.

MATERIALS AND METHODS

Cells and Peptides. A.E7 is a Th1, $CD4^+$ T-cell clone specific for the fragment 81–104 of pigeon cytochrome *c* (PCC) bound to the I-E^k class II MHC molecule (22), which was grown as reported (16). APCs used with agonist ligand PCC(88–104) were DCEK L-cell transfectants (23) selected to express high levels of EαEβ^k and very low levels of B7.1 (24). These cells lack appreciable costimulatory activity after fixation. Antigen presenting cells used for anergy induction with partial agonist ligand moth cytochrome *c* [MCC(93–103)] with position 97 changed to I {MCC(93–103)[97I]} were unsorted DCEK-Hi-7 cells expressing significant levels of B7.1 (16). Peptides were synthesized by standard fluorenylmethoxycarbonyl (Fmoc) chemistry by the National Institute of Allergy and Infectious Diseases Peptide Facility and purified by HPLC to >95% purity.

Anergy Induction and IL-2 Rescue. Anergy induction by agonist on noncostimulatory APCs was achieved by incubating 10^6 A.E7 T cells with 10^6 fixed DCEK cells together with 10^{-5} M or 10^{-4} M PCC(88–104) in a final volume of 1.5 ml. The DCEK APCs used in these primary culture were either mitomycin C-treated (control) or fixed with 0.93% paraformaldehyde and extensively washed before use. Anergy induction by partial agonist on live APCs was achieved using the same cell numbers and using MCC(93–103)[97I] instead of PCC(88–104). After 24 hr of incubation with APC and peptide, T cells were collected by two rounds of magnetic depletion of APC, followed by centrifugation with Ficoll–Hypaque (Pharmacia). Viable T cells were plated in 2 ml and rested for 3–7 days. After this rest, T cells were harvested, counted, and stimulated (3×10^4 cells per well) with different concentrations of agonist peptides [PCC(88–104) or MCC(93–103)] presented by live DCEK cells. The effect of IL-2 on the induction of anergy was evaluated by adding IL-2 containing supernatant (Collaborative Research) at 1:4 final dilution or recombinant mouse IL-2 (PharMingen) at 10 units/ml or 30 units/ml to the 24-hr induction culture. The IL-2 remained throughout this 24 hr and was removed during the isolation of viable cells for the rest culture.

Effector Response Assays. Cell proliferation was measured by [^{3}H]thymidine uptake during the last 18 hr of a 48-hr culture. IL-2 production was measured by ELISA (25). T-cell responses to exogenous IL-2 were measured after the rest period by incubating 3×10^4 cells in IL-2-containing medium (RAT-T Stim; Collaborative Research) at a final dilution of 1:4 or in 20 units of recombinant mouse IL-2 per ml (PharMingen), followed by analysis of [^{3}H]thymidine uptake during the last 18 hr of a 48-hr culture.

TCR-Induced Tyrosine Phosphorylation Assays. Immunoblot analysis for protein tyrosine phosphorylation using cell lysates or anti-CD3ε immunoprecipitates was performed as described (16). The following antibodies were used: 4G10, a mouse IgG2b monoclonal antibody to phosphotyrosine (Upstate Biotechnology); and 500A2, a hamster antibody against the mouse CD3ε chain (PharMingen). Cell lysate results were generated from 6×10^5 cell equivalents per lane. Immunoprecipitations were performed using 10^7 cells per group. The effect of IL-2 on early TCR-induced tyrosine phosphorylation was determined by stimulation of T cells in the presence of IL-2 containing supernatant (Collaborative Research) at a 1:4 dilution or in 10 units of recombinant mouse IL-2 per ml (PharMingen), added to the culture wells with APCs and peptide before addition of the T cells. Tyrosine phosphorylation was then determined for proteins in whole cell lysates or in anti-CD3 immunoprecipitates as described (16).

RESULTS

Anergy Induction by Fixed (Noncostimulatory) Cells Bearing Agonist Ligand or Live (Costimulatory) Cells Bearing Partial Agonist Ligand. To investigate the role of altered signaling in anergy induction, a cloned PCC-specific T cell (22) previously employed in studies of anergy induced by typical agonists was used to identify a variant ligand with the partial agonist properties described for some hemoglobin peptides (15). A MCC(93–103) peptide with a tyrosine to isoleucine substitution at protein position 97 {MCC(93–103)[97I]} was found to have the desired partial agonist properties—specifically, the ability to induce the previously described pattern of variant TCR-associated tyrosine phosphorylation (15, 16) coupled with a failure to induce detectable IL-2 secretion in the presence of viable APCs (data not shown). We then compared anergy induction upon exposure to agonist ligand on nonviable APCs with the development of unresponsiveness due to recognition of this variant ligand borne by live presenting cells expressing constitutively high levels of the costimulatory molecule B7.1.

As expected, cells of the Th1 clone A.E7 did not proliferate in response to the agonist peptide PCC(88–104) presented by chemically fixed (costimulation-deficient) I-E^k-bearing cells (Fig. 1*A*). They also did not proliferate significantly to the variant ligand MCC(93–103)[97I] presented by live costimulatory APCs (Fig. 1*B*). In agreement with previous studies, exposure of the A.E7 cells to the agonist combination PCC(88–104)/I-E^k on fixed APCs rendered the T cells anergic (10, 11, 20, 21). After a 3- to 7-day rest following ligand exposure, this cell population showed no proliferative (Fig. 1*C*) or IL-2 secretory responses (Fig. 1*E*) when confronted with the same agonist ligand on live, costimulation-positive APCs. In agreement with the observations of Sloan-Lancaster *et al.* (14, 15), the nonstimulatory 97I variant of the MCC(93–103) ligand also rendered the T cells unresponsive to subsequent presentation of PCC(88–104)/I-E^k agonist on viable cells, as measured by either proliferation or IL-2 production (Fig. 1 *D* and *F*). The unresponsiveness of A.E7 after exposure to either of these ligand/APC combinations was not due to induction of cell death, as the cells were viable after the rest period and showed good proliferation to exogenous IL-2 (Fig. 1 *G* and *H*).

Distinct Patterns of Early TCR-Induced Phosphorylation Are Induced Following Exposure to Either Agonist Ligand on Fixed Cells or Partial Agonist on Live Cells. These results show that phenotypically similar states of unresponsiveness affecting proliferation and IL-2 secretion can be induced in the same T-cell clone under two distinctly different conditions of TCR occupancy and costimulation. To examine whether a common pattern of altered TCR signaling led to the anergic state in both cases, the early tyrosine phosphorylation events occurring using live cells with the partial agonist and either live or fixed cells with full agonist were studied (Fig. 2). As previously reported for other cytochrome (16) or hemoglobin peptides (15), live cell presentation of the 97I variant ligand induced accumulation of p21 phospho-ζ, without significant accumulation of either p23 phospho-ζ, phosphorylated CD3ε, or phosphorylated ZAP-70 (Fig. 2*A*). In marked contrast, however, agonist ligand induced a distinct pattern of total substrate phos-

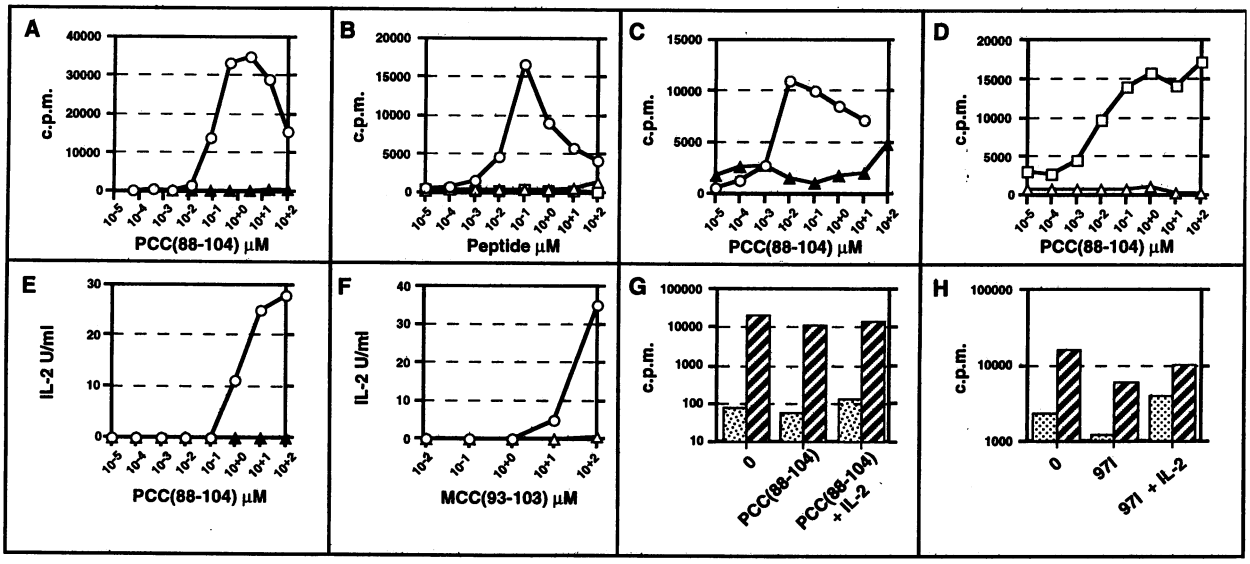

FIG. 1. Induction of T-cell anergy by agonist or partial agonist ligand. (*A*) Response of resting A.E7 T cells to PCC(88–104) agonist presented by live (○) or fixed (▲) APCs. (*B*) Response of resting A.E7 T cells to MCC(93–103) agonist (○), its analogue 97I (△), or control peptide 102I (□) presented by live APCs. (*C*) T cells exposed to PCC(88–104) agonist on fixed APCs are unable to proliferate in response to agonist presented by viable APCs. T cells were rendered anergic by a 24-hr exposure to PCC(88–104) on fixed APC, followed by 7 days of rest. T cells were harvested and challenged with PCC(88–104) on live APCs. ○, Response of T cells that were initially exposed to fixed APCs with no antigen. ▲, Response of T cells initially exposed to PCC(88–104) on fixed APC. (*D*) T cells exposed to 97I partial agonist on live APCs are unable to proliferate in response to PCC(88–104) agonist on live APCs. □, T cells that were initially exposed to a control peptide 102I presented by live APCs. △, Response of T cells initially exposed to 97I on live APCs. (*E*) T cells exposed to agonist on fixed APCs (▲) fail to produce IL-2 in response to PCC(88–104) presented by live APCs. ○, Responses of cells initially cultured with fixed APC without peptide. (*F*) T cells exposed to partial agonist 97I on live APCs (△) fail to produce IL-2 in response to MC(93–103) agonist presented by live APCs. ○, Represent responses of cells initially exposed to live APCs without peptide. (*G*) T cells anergized by agonist on fixed APC are responsive to exogenous IL-2. Bars show proliferation of cells initially cultured without or with antigen and fixed APC after a 7-day rest period. Dotted fill shows results without added IL-2; diagonal fill shows results with IL-2 added at the time of reculture after rest. The group labeled PCC(88–104) + IL-2 on the *x*-axis had IL-2 added during the 24-hr initial antigen exposure before rest. (*H*) T cells anergized by partial agonist 97I on live APCs are responsive to exogenous IL-2. Bars show proliferation of cells initially cultured without or with antigen and live APCs after a 7-day rest period. Dotted fill shows results without added IL-2; diagonal fill shows results with IL-2 added at the time of reculture after rest. The group labeled 97I + IL-2 on the *x*-axis had IL-2 added during the 24-hr initial antigen exposure before rest.

phorylation that was similar whether live or fixed presenting cells were used (Fig. 2*B*). Furthermore, the previously reported agonist pattern of nearly equal amounts of both p21 and p23 phospho-ζ, good CD3ε phosphorylation, and TCR-associated phosphorylated ZAP-70 was observed under both stimulatory (live cell) and anergy-inducing (fixed cell) conditions (Fig. 2*C*). These data make it clear that the variant pattern of early TCR-associated signaling events seen using

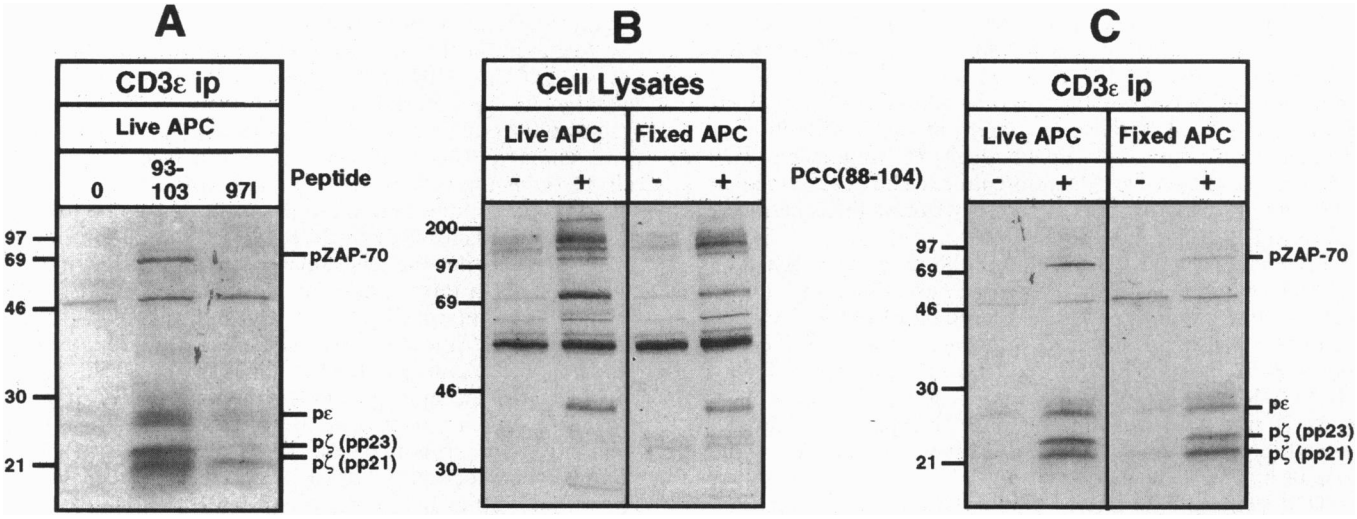

FIG. 2. Tyrosine phosphorylation of TCR-associated proteins during induction of T-cell anergy by agonist ligand on fixed APC or partial agonist on live APCs. T-cell lysates were prepared after stimulation with MCC(93–103) or 97I on live cells for 10 min (*A*) or stimulation of T cells with PCC(88–104) presented by live and fixed APCs for 10 min (*B* and *C*, respectively). (*A*) Anti-CD3ε immunoprecipitates from T cells exposed to MCC(93–103) agonist or 97I partial agonist on live APCs were immunoblotted for phosphotyrosine. (*B*) Anti-phosphotyrosine immunoblot of whole cell lysates from T cells exposed to live or fixed APC presenting PCC(88–104). (*C*) Anti-phosphotyrosine immunoblot of proteins immunoprecipitated by anti-CD3ε antibody from the same cell lysates shown in *B*. Molecular size markers are on the left of each panel. The positions of phosphorylated ζ, CD3ε, and ZAP-70 are indicated on the right of the panels showing blotting of anti-CD3ε immunoprecipitates.

partial agonists is neither necessary for, nor uniquely associated with, the development of anergy.

Prevention/Reversal of Anergy Induction Using IL-2 Without Changes in the Agonist or Partial Agonist Signaling Patterns. Previous studies have shown that anergy induction by agonist in the absence of costimulation can be either prevented or reversed by exposure to cytokines whose receptors use the common γ chain (17–19). In agreement with these prior reports, inclusion of IL-2 during the first culture of A.E7 with fixed presenting cells and agonist ligand substantially prevented the unresponsive state that otherwise occurs (Fig. 3*A*). The reversal under these limited conditions of IL-2 exposure (present during the first 24 hr of ligand engagement, then withdrawn during the rest period) was incomplete but significant and reproducible in several experiments of this type. To examine the possibility that the altered signaling pattern occurring with partial agonist recognition induced a distinct and perhaps irreversible state of intracellular inactivation as compared with agonist ligand on fixed APCs, the effect of IL-2 addition was also tested with variant ligand presentation by live APC. As with agonist, the anergic state resulting from exposure to 97I on live cells could be prevented by inclusion of IL-2 during the initial TCR engagement phase (Fig. 3*B*). In these experiments with partial agonist, the ability of IL-2 to rescue responsiveness was generally more pronounced than with agonist ligand.

The effects of IL-2 addition on TCR-associated phosphorylation events observed under both anergy-inducing conditions were also evaluated. No changes in the distinctly different patterns associated with agonist or partial agonist ligand were observed due to the presence of IL-2 at the time of ligand exposure (Fig. 4).

DISCUSSION

Substantial interest has recently been generated by the discovery of TCR ligands with the properties of partial agonists or antagonists (reviewed in refs. 26–31) and the demonstration that such ligands appear to induce a pattern of early TCR-related tyrosine phosphorylation distinct from that seen across a wide concentration range of full agonist ligand (15, 16). In particular, the correlation between a variant pattern of ζ chain phosphorylation and ZAP-70 activation and the induction of anergy by altered peptide ligands (15) has led to the intriguing hypothesis that signaling T cells in this aberrant manner is directly linked to induction of the unresponsive state termed anergy. Because of other results, however, suggesting that anergy could also be induced by typical agonist ligands and limited data indicating that normal TCR-dependent signaling occurred under the conditions necessary to achieve anergy using these ligands (11, 20, 21), it was clear that an analysis of whether variant pattern of TCR signaling was directly and necessarily linked to induction of unresponsiveness was required. The data reported here are inconsistent with a model in which the altered TCR tyrosine phosphorylation events seen with partial agonists are either necessary for induction of, or irreversibly commit a T cell to, the anergic state. Rather, it appears to be a common failure to produce enough IL-2, though by different means, that leads to the shared outcome of induced unresponsiveness using agonist and partial agonists under suitable presentation conditions. Our data thus support earlier conclusions drawn from functional studies using only agonist ligand (17, 18) and extend them by providing both functional and biochemical data concerning anergy induction in the same T cell-mediated by both agonists and partial agonists in the presence and absence of IL-2. Although the present data have been obtained using a single cloned T-cell line, it has been previously demonstrated that both the functional and signaling properties of this cell line are characteristic of numerous other cloned lines and/or primed T-cell populations and thus are likely to reflect the behavior of many antigen-experienced T cells exposed to TCR ligands under similar circumstances.

In the case of agonist ligand, interference with the critical event of IL-2 production arises from the absence of adequate costimulatory signals, especially those from the CD28/CD80 or CD86 interactions (12, 13, 32–35). As concluded from previous studies of more distal events, such as Ca^{2+} elevation and phosphoinositide production, the analysis here of proximal tyrosine phosphorylation events is consistent with agonist ligand on fixed cells providing the usual set of intracellular TCR-derived signals that would, if such CD28-dependent costimulation were available, result in full T-cell effector responses and retention of a responsive state. In the case of partial agonists, even when costimulation is present, altered signaling leads to suboptimal or absent IL-2 production. This

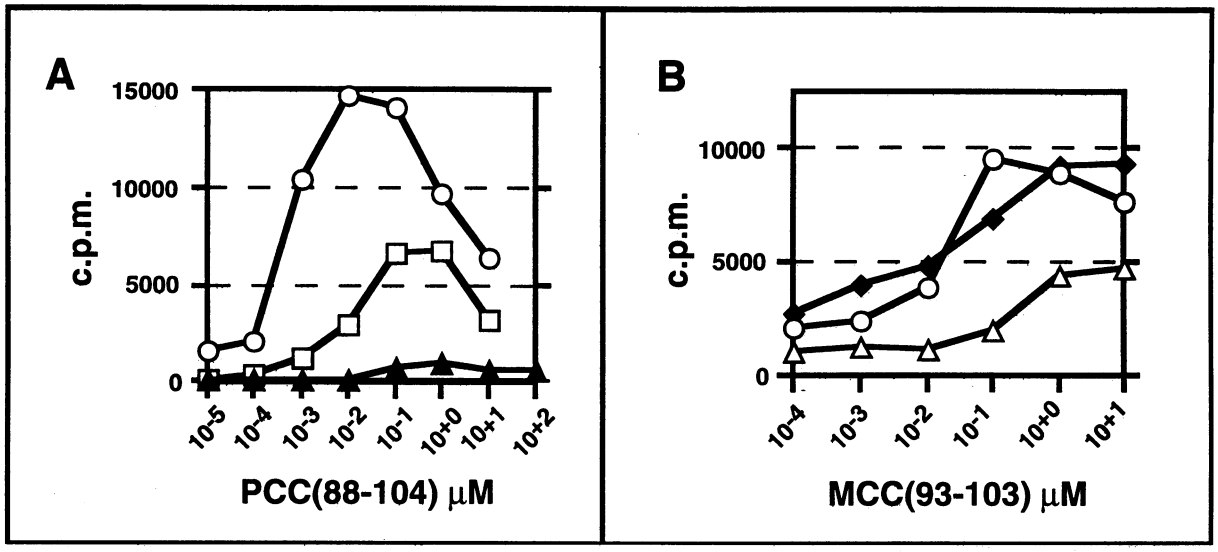

FIG. 3. IL-2 rescues cells from anergy induced by either agonist on fixed APC or partial agonist on live APCs. (*A*) Responses are shown for A.E7 cells initially cultured for 24 hr with fixed cells and no peptide antigen (○), fixed cells and PCC(88–104) agonist peptide without IL-2 (▲), or fixed cells, PCC(88–104), and IL-2 (□), then rested and restimulated with PCC(88–104) after 7 days. (*B*) Responses are shown for A.E7 cells initially cultured for 24 hr with live cells and no peptide antigen (○), live cells and 97I partial agonist peptide without IL-2 (△), or live cells, 97I, and IL-2 (◆), then rested and restimulated with PCC(88–104) after 7 days.

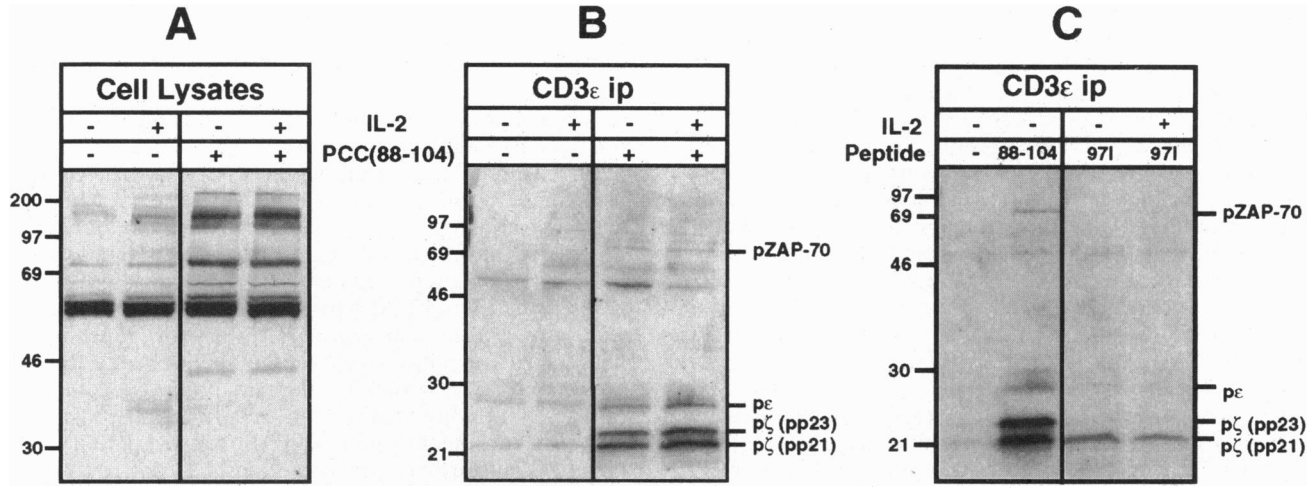

FIG. 4. IL-2 does not detectably change the early TCR-induced tyrosine phosphorylation patterns elicited by agonist or partial agonist ligands. (*A*) Phosphotyrosine immunoblot of proteins from A.E7 cells exposed for 10 min to fixed APCs with or without agonist PCC(88–104) peptide in the presence or absence of IL-2. (*B*) Phosphotyrosine immunoblot of proteins precipitated from the lysates shown in *A* after precipitation with anti-CD3ε antibody. (*C*) Phosphotyrosine immunoblot of proteins precipitated with anti-CD3ε antibody from lysates of A.E7 cells exposed for 10 min to live APCs in the presence or absence of partial agonist 97I with or without IL-2.

presumably arises from the very low level or the absence of certain biochemical events that are normally complemented by costimulatory signals arising from CD28 engagement, such as activation of Jun N-terminal kinase (36).

It has been demonstrated that an antibody to the common γ chain of the IL-2 receptor can prevent anergy induction in human T-cell clones under conditions not giving rise to multiple rounds of cell division (19), while others (17, 18) observed that extensive growth in IL-2 was necessary for optimal reversal of an existing anergic state in mouse T cells. Together these data suggest that IL-2 may act at two levels. First, it may inhibit development of anergy through signals from the IL-2 receptor provided contemporaneously with or immediately following TCR engagement, and second, it may reverse an established anergic state through subsequent cell cycle-dependent events that occur late, long after the cessation of TCR signaling. Such events may include an alteration in CD2 structure or membrane associations that has been reported to correlate with reversal of anergy in human cells (37) or a change in TCR-associated kinase function (38). Consistent with both these postulated modes of action, in the present experiments, interference with anergy induction or maintenance was seen using either IL-2 exposure contemporaneously with TCR engagement during the 24-hr induction period, followed by rest in the absence of either TCR ligand or IL-2, and also with IL-2 addition delayed until removal of the TCR ligand at 24 hr, then present throughout the rest period (unpublished observations).

The common abilities of both agonist and partial agonist ligands to induce a phenotypically similar state of anergy and of IL-2 to prevent this anergy induction in each case strongly suggest that a similar molecular mechanism underlies the induction of the unresponsive state in both circumstances. This further implies that at least some signals arising from TCR engagement by the two ligand types are likely to be the same, regardless of the superficial differences in TCR-associated tyrosine phosphorylation seen upon immunoblotting. Anergy induction appears to involve the Ca^{2+}/calmodulin/calcineurin pathway in both situations, as it is blocked by cyclosporin A when using either agonists (39) or partial agonists (14) and can be partially mimicked by ionomycin (40). Recent data suggest that ZAP-70 kinase may be critical for the induction of Ca^{2+} responses by T cells (41). Because anergy induction by partial agonists appears to be dependent on such Ca^{2+} signals, the novel pattern of tyrosine phosphorylation induced by partial agonists may not reflect a stringent block preventing all phosphorylation and enzymatic activation of ZAP-70, as originally proposed based on phosphotyrosine immunoblotting data. Rather, this pattern may be generated by short-lived ZAP-70 activation due to transient nonreceptor *src* family kinase activity induced by either brief TCR engagement with variant peptide/MHC molecule ligands (27–29, 31, 42) or by short-lived cell–cell interactions (43). This would allow the partial agonist-stimulated cells to receive at least limited downstream signals derived from such transient activation of ZAP-70, including a rise in intracellular Ca^{2+} as predicted by the cyclosporin A inhibition data. Some partial agonists may fail to generate an adequate level of this weak or transient signal, accounting for the variation in anergy induction seen in the hemoglobin model (15) and observed by us for different cytochrome peptides (J.M. and R.N.G., unpublished observations). These transient signals may give rise to the same unknown molecular block that is distal to early TCR-linked *src* kinase activation but proximal to activation of *ras*, as recently reported to be characteristic of T cells made anergic by agonist ligands (44). This block may represent the first phase of a normal homeostatic process that regulates the mitogen-activated protein kinase (MAPK) pathway (45) and that is not reversed due to the failure of the cells to receive the "expected" IL-2 receptor signal.

Aside from these signaling considerations, the present data bear on our understanding of the evolution of the self-tolerant state and on the use of partial agonist ligands for therapy of autoimmune disease. In the case of tolerance induction, self-peptide partial agonists presented at very high ligand density following inflammatory tissue damage and increased antigen processing (46) might give rise to effector responses rather than anergy, if enough IL-2 were made available through foreign antigen activation of neighboring T cells. Likewise, during attempts to use synthetic partial agonists to anergize autoreactive T cells, such paracrine IL-2 production by anti-pathogen T cells (47) could rescue the autoreactive cells exposed to high levels of the administered ligand from anergy. This might lead to increased effector responses and aggravate disease, rather than reduce it. It thus remains for further experiments and clinical trials to determine which will predominate: variant ligand-induced T-cell inactivation, or augmented function due to ambient IL-2 or other proliferative signals present in an active inflammatory site.

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