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# Increased Threshold for TCR-Mediated Signaling Controls Self Reactivity of Intraepithelial Lymphocytes[1]

**Sarah R. Guehler,* Rosalynde J. Finch,† Jeffrey A. Bluestone,‡ and Terrence A. Barrett[2]***

**To examine the effect of self Ag on activation requirements of TCR-$\alpha\beta$ intestinal intraepithelial lymphocytes (IELs), we utilized the 2C transgenic (Tg) mouse model specific for a peptide self Ag presented by class I MHC, H-2L$^d$. CD8$\alpha\alpha$ and CD4$^-$CD8$^-$ IELs from syngeneic (H-2$^b$, self Ag$^-$) and self Ag-bearing (H-2$^{b/d}$, self Ag$^+$) strains were examined for their ability to respond in vitro to P815 (H-2$^d$) cell lines expressing the endogenous antigenic peptide, p2Ca. Proliferation, cytokine production, and CTL activity were elicited in IEL T cells isolated from self Ag$^-$ H-2$^b$ mice when stimulated with P815 cells expressing basal levels of self Ag. These responses were enhanced following the addition of exogenous p2Ca peptide and ectopic expression of the costimulatory molecule, B7-1. By comparison, IEL from self Ag-bearing mice failed to respond to basal levels of self Ag presented by P815 cells even in the presence of B7-1-mediated costimulation. However, the addition of increasing amounts of exogenous p2Ca peptide induced a response from the in vivo "tolerized" T cells. These results suggest that exposure to self Ag in vivo increased the threshold of TCR activation of Ag-exposed self-reactive IELs. The dependence of increased signal 1 to activate self-reactive IELs suggests a defect in TCR signaling that may maintain self tolerance in vivo. These data suggest that conditions that overcome signal 1 IEL defects may initiate autoreactive responses in the intestine.** ***The Journal of Immunology*****, 1998, 160: 5341–5346.**

The consequence of self tolerance for intraepithelial lymphocytes (IEL)[3] is distinct from T cells in peripheral lymphoid tissue. Thymic deletion predominates for self-reactive peripheral T cells, whereas nondeletional mechanisms operate for IELs. For example, in mice expressing the minor lymphocyte stimulatory (Mls)-1$^a$ and mouse mammary tumor virus (MMTV) self Ags, a subset of potentially self-reactive V$\beta$6$^+$-, V$\beta$8.1$^+$- and V$\beta$11$^+$-expressing IELs is present despite the thymic deletion of peripheral T cells (1, 2). Likewise, Ag-specific self-reactive IEL are present in male H-Y TCR Tg mice (3). In both instances, potentially self-reactive CD8$\alpha\beta$-expressing IELs were deleted, whereas CD8$\alpha\alpha$ and CD4$^+$CD8$^+$-expressing IELs persisted. Although the CD8$\alpha\alpha$ IELs were functionally unresponsive to activating stimuli, it was unclear whether this was due to functional tolerance or developmental immaturity (4–6).

In previous studies, we showed that the 2C TCR Tg model was similar to the other Tg models. Lymphoid T cells expressing Tg$^+$ TCR were deleted when Ag (in this case allogeneic H-2L$^d$ plus self peptide) was expressed by the mice. By comparison, CD8$\alpha\beta$ Tg$^+$ IELs were not present, whereas CD8$\alpha\alpha$ and CD4$^-$CD8$^-$ Tg$^+$ IEL persisted with the same frequency as Tg$^+$ IELs from self Ag$^-$ mice (7). Interestingly, the exposure to self Ag induced an activated phenotype of Thy-1$^{dul/-}$ and CD45R/B220$^+$, and immune deviation of Tg$^+$ IEL from TC1- to TC2-like IEL subsets. Thus, tolerance of IELs in the 2C Tg model involved deletion of CD8$\alpha\beta^+$ Tg T cells in the periphery and intestine. For persisting CD4$^-$CD8$^-$ and CD8$\alpha\alpha$-expressing IEL subsets, self tolerance involved functional differentiation to less inflammatory cell types.

In the present study, we determined the activation requirements for IELs from self Ag-bearing 2C Tg$^+$ mice. P815 or B7-1-bearing P815 mastocytoma cells were used as APCs to examine the functional responses to antigenic peptide (signal 1) as opposed to costimulatory signals (signal 2). The results suggest that signal 1, not signal 2, defects controlled self reactivity in self Ag$^+$ mice.

## Materials and Methods

### *Mice*

Adult H-2$^b$ and H-2$^{b/d}$ Tg mice (self Ag$^-$ and self Ag$^+$, respectively) were generated by breeding a 2C Tg$^+$ H-2$^b$ male (a gift from Dr. Dennis Loh, Nippon Research Center, Kanagawa, Japan) to either C57BL/10 or BALB/c females obtained from National Cancer Institute (Frederick, MD) animal stock. Animals were raised under specific pathogen-free conditions in the Veterans Administration Lakeside Medical Center, Medical Science Building (Chicago, IL).

### *Culture medium*

Culture medium consisted of DMEM, 10 mM HEPES, 5% FCS, 2-ME, glutamine, antibiotics, and nonessential amino acids, as previously described (8).

### *Cell isolation*

Intestines were removed from 6- to 8-wk-old mice and IELs isolated as previously described (8) with minor modifications. Briefly, small intestines were removed and flushed with cold PBS. Intestines were opened longitudinally and cut into 1-cm pieces. After brief vortexing and multiple rinses with cold PBS, the pieces were resuspended in 50 ml digestion buffer containing 10% newborn calf serum (Life Technologies, Grand Island, NY), 0.3 mg/ml dithioerythritol (Life Technologies), with 5 mM EDTA in PBS. Pieces, suspended in this buffer, were gently agitated at 40 to 50 revolutions/min in a closed 75-ml digestion flask (Fisher Scientific, Itasca, IL) with a stir bar at 37°C for 40 min. Pieces were washed with cold PBS

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[3] Abbreviations used in this paper: IEL, intestinal intraepithelial lymphocyte; Tg, transgenic; FCM, flow cytometry.

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and the supernatant collected and pelleted. Pellets were resuspended in 5% DMEM and kept at 4°C for over 2 h. The cells were resuspended in 50% Percoll (Pharmacia, Piscataway, NJ) and 0.3 mg/ml DTT, layered onto a discontinuous Percoll gradient (75% density) and centrifuged for 20 min at 20°C at 400 × *g*. The cells concentrated at the interface of the 50% and 75% layers were then pipetted off and washed in 4 vol of PBS. The purity of Tg IELs within preps was assessed by FCM on the basis of forward angle and 90° light scatter, as well as using fluorochrome-coupled Tg clonotypic mAb, 1B2.

### *Abs, three-color immunofluorescence, and immunofluorescence analysis*

The following mAbs coupled to FITC, phycoerythrin, or biotin were used: anti-CD8$\alpha$, anti-CD8$\beta$ (PharMingen, San Diego, CA) and anti-Tg TCR mAb, 1B2 (a gift from Dr. Dennis Loh) (9). Biotin-labeled Abs were followed by streptavidin-CyChrome or streptavidin-phycoerythrin (PharMingen). Dead cells were excluded from analysis on the basis of forward and side angle scatter and in some cases by propidium iodide (Sigma Chemical Co., St. Louis, MO). Approximately $5 \times 10^5$ cells were stained per sample for 20 min with a concentration of mAb titered to maximize specific staining and limit background. A total of 10,000 gated events were collected for analysis. Acquisition of FCM data was performed on a FACScan, and cell sorting was performed on a FACStar$^{Plus}$ (Becton Dickinson, Mountain View, CA). Data were analyzed using the CellQuest program (Becton Dickinson).

### *Negative depletion of CD8$\alpha\beta$ IELs*

H-2$^b$ IELs were incubated at 4°C with anti-CD8$\beta$ (53–5.84) (10, 11) for 30 min, washed three times with cold DMEM, and resuspended with sheep anti-rat IgG Ab-coated magnetic beads at a 3:1 ratio following the manufacturer's directions (Dynal, Lake Success, NY). After a 30-min incubation at 4°C with slow mixing, beads were collected by magnet, negatively selecting the CD8$\alpha\beta$ population. Any remaining CD8$\beta^+$ cells were depleted a second time with fresh beads and magnetic separation. Purity was assessed by FCM goat anti-rat IgG FITC (Kirkegaard and Perry Laboratories, Gaithersburg, MD).

### *Generation of murine B7-1 expression construct, stable transfection of murine B7-1 into P815 cells*

Murine B7-1 was cloned into the eukaryotic expression vector pNA′ (12), a modified form of pHbAPr-neo (13) that contains the human $\beta$-actin promoter and confers resistance to neomycin. The cDNA sequence for murine B7-1 was initially removed as an *Eco*RI fragment and inserted into the *Eco*RI site of pcDNA3(Invitrogen, Carlsbad, CA). A subclone containing B7-1 sequence in the correct orientation was selected; B7-1 was removed with a *Kpn*I, *Xba*I digest, and ligated into pNA′ that had been digested with *Kpn*I and *Xba*I. Resulting subclones were screened for correct orientation relative to the $\beta$-actin promoter, and one subclone was selected for transfection into P815. pNA′/B7-1 was linearized with *Sca*I and transfected into P815 cells by electroporation. pNA′/B7-1 transfectants were selected on 1 mg/ml G418 (Gemini Bioproducts, Calabasa, CA). Resulting antibiotic-resistant cells were screened for B7-1 expression by FCM using FITC-conjugated CD80 (1G10) Ab (PharMingen). To select for high expression of surface B7-1 by P815 cells, bulk populations of transfected cells were sorted by FCM and plated at a density of 1 cell/well. Wells with growth after 2 wk were then rescreened by FCM, and subclones with the desired levels of B7-1 and expression were selected.

### *Proliferation assays*

For each condition, $3 \times 10^4$ irradiated P815 mastocytoma cells were cocultured with $1 \times 10^5$ responder Tg IELs. Tg numbers were determined by flow cytometry analysis, to normalize for Tg$^+$ expression in culture, in 96-well round-bottom microtiter plates in triplicate. Exogenous p2Ca peptide (sequence LSPFPFDL (14, 15) (Bio-Synthesis, Lewisville, TX)) was added to some experiments using H-2$^d$ APC. Unless otherwise noted, p2Ca peptide was used at 1.0 $\mu$g/ml concentration. Exogenous human rIL-2 (50 U/ml) (Genzyme, Cambridge, MA) was added when indicated on day 1 of culture. At 48 h, cultures were pulsed for 16 to 18 h with [$^3$H]-labeled thymidine (1 mCi/well). Cells were harvested and analyzed with a liquid scintillation counter (Packard International, Meriden, CT).

### *Lymphokine assays*

Isolated IELs were cultured in 96-well plates as described above. After 48 h, supernatants were harvested and analyzed for IL-2 and IFN-$\gamma$, using murine cytokine ELISA MiniKits (Endogen, Cambridge, MA). The sensitivities of these ELISAs were as follows: <10 pg/ml for IL-2, and <100 pg/ml for IFN-$\gamma$.

### *Measurement of cytolytic activity*

Cytolytic activity of IEL was measured using a standard lysis assay as previously described (16) with minor modifications. Purified IELs obtained from the isolation procedure were assayed for cytolytic activity against ($^{51}$Cr)sodium chromate-labeled P815 (DBA/2 mastocytoma) target cells alone or in the presence of 1.0, 0.1, or 0.01 $\mu$g/ml p2Ca peptide. Target cells were labeled for 1.5 h, washed, then plated with serial dilutions of effector cells in 96-well round-bottom microtiter plates for 4 or 15 h at 37°C. Effector/target ratios were based on $2 \times 10^3$ target cells/well. Percent specific lysis was determined as 100 × [(c.p.m. test released − c.p.m. control released)/(c.p.m. maximum released − c.p.m. control released)]. Spontaneous release was less than 25% for all experiments.

## Results

### *Proliferative responses of IEL populations*

To compare the activation requirements for IEL from self Ag-bearing (self Ag$^+$, H-2$^{b/d}$) mice and the corresponding CD4$^-$CD8$^-$ and CD8$\alpha\alpha$ subsets from syngeneic (self Ag$^-$, H-2$^b$) Tg$^+$ IELs, proliferative responses were assessed. In previous studies, we have shown that similar populations (CD8$\beta$-depleted) of Tg$^+$ IELs from self Ag$^+$ mice proliferated at lower levels compared with self Ag$^-$ mice in response to H-2$^d$ splenic APC. This defect could be a result either of defective signaling via the TCR complex or of altered costimulation. Thus, the IELs were examined for their ability to proliferate in the presence of increased signal 1 (TCR-mediated) by adding additional Ag, p2Ca peptide, to the L$^d$-bearing

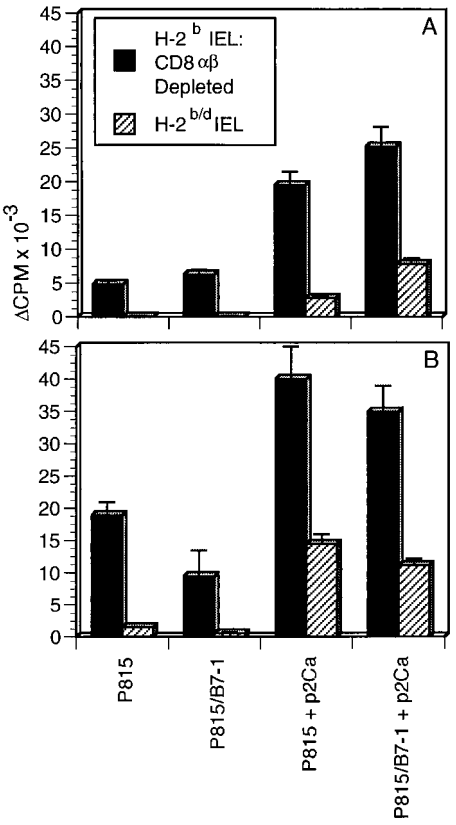


**FIGURE 1.** Differential proliferative response of H-2$^b$ and H-2$^{b/d}$ IELs. IEL responses were compared without (*A*) and with (*B*) 50 U/ml IL-2. Proliferative responses were measured for CD8$\alpha\alpha$ and CD4$^-$CD8$^-$ Tg$^+$ IELs from both H-2$^b$ (solid bars) and H-2$^{b/d}$ (hatched bars) mice in response to P815 or B7-1-transfected P815 (P815/B7-1) APC alone or with added antigenic p2Ca peptide. All measurements were performed in triplicate and the data expressed as the mean, with an SE < 15%. Data shown are representative of four experiments.

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APCs or increasing signal 2 by the addition of additional CD28 signaling or exogenous IL-2. As shown in Figure 1, IEL responses from self $Ag^-$ mice were induced by basal levels of self Ag expressed on P815 cells and were enhanced with added peptide self Ag, B7-1-mediated costimulation (Fig. 1*A*), or IL-2 (Fig. 1*B*). Potential transcostimulation by B7 expression on IELs did not influence their response, since inclusion of CTLA4Ig with P815 plus peptide self Ag did not diminish IEL proliferative response (data not shown). In contrast, IELs from self $Ag^+$ mice did not respond to basal levels of self Ag but required the presence of increased peptide self Ag to proliferate (Fig. 1*A*). Addition of IL-2 and p2Ca self Ag to parental P815 cells induced significant proliferative responses for $Tg^+$ IEL from self $Ag^+$ mice, although addition of IL-2 without peptide self Ag did not induce proliferation (Fig. 1*B*)(stimulation index (SI) = 21 compared with 3 respectively, $p < 0.05$). No specific synergistic effects of IL-2 were detected with added costimulation, although proliferative responses were increased overall. Thus, $Tg^+$ IEL from self $Ag^-$ mice responded to increased signal 2, whereas increased costimulation had no effect on $Tg^+$ IEL responses from self $Ag^+$ mice unless TCR signaling was enhanced with p2Ca peptide.

*Effect of signal 1 and signal 2 on IEL survival*

Costimulation has been shown to enhance survival of activated $CD8^+$ T cells, when examined relatively late (96 h) after activation, likely through prolonged cell survival by CD28-mediated upregulation of the survival gene Bcl-xL (17). Thus, the effects of costimulation may not be enhanced activation but prolonged expansion. Therefore, the effects of enhanced signal 1 and/or signal 2 on IEL survival were examined for $Tg^+$ IEL from self $Ag^-$ and self $Ag^+$ mice by assessing the number of live $Tg^+$ cells after stimulation with control or transfected P815 cells with or without added p2Ca peptide. Stimulation of $Tg^+$ IEL from self $Ag^-$ mice with B7-transfected P815 cells increased $Tg^+$ IEL survival in the presence or absence of exogenous peptide (10-fold and 5-fold, respectively (Fig. 2). As predicted by the proliferation results, fewer viable $Tg^+$ T cells were recovered from cultures of IEL from

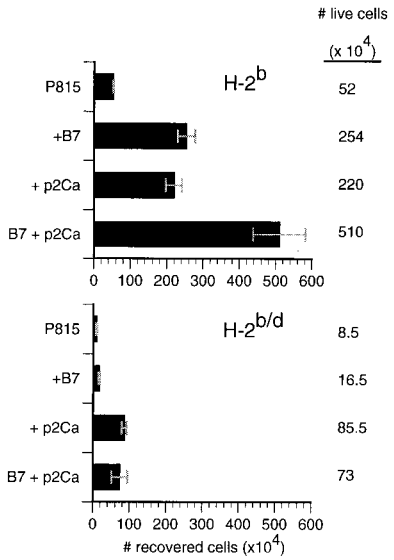


**FIGURE 2.** Increased TCR signaling enhances IEL recovery. Initial stimulation of $5 \times 10^5$ $CD8\alpha\alpha$ and $CD4^-CD8^-$ $Tg^+$ IELs from H-$2^b$ (*A*) and H-$2^{b/d}$ (*B*) mice were cultured with P815, P815 + p2Ca peptide, P815/B7-1, or P815/B7-1 + p2Ca peptide. Live $Tg^+$ IELs recovery on day 4 is shown. All conditions were performed in triplicate and the data are expressed as a mean, with SE < 10%.

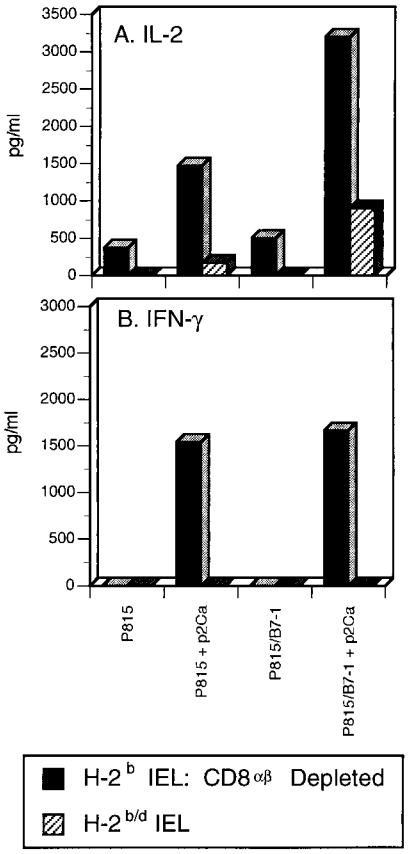


**FIGURE 3.** Cytokine response of fresh IEL populations. IELs were cultured with P815 + p2Ca peptide, P815/B7-1, or P815/B7-1 + p2Ca peptide. Culture supernatant was collected at 48 h for the conditions indicated, and levels of IL-2 (*A*) and IFN-γ (*B*) protein measured by ELISA.

self $Ag^+$ mice activated with P815 as compared with IELs from self $Ag^-$ mice or cultured in the absence of Ag-pulsed APC. Although addition of B7 increased self $Ag^+$ $Tg^+$ IEL survival, numbers failed to reach "baseline" levels detected in unstimulated wells. Interestingly, addition of p2Ca in combination with the additional costimulatory signal increased self $Ag^+$ $Tg^+$ IEL survival by 10-fold compared with cells cultured with P815 alone. Thus, $Tg^+$ IEL survival in self $Ag^+$ mice depended largely on increasing signal 1 and pointed to a defect in the TCR pathway.

*Cytokine response of fresh IEL populations*

IL-2 and IFN-γ levels were assessed for $Tg^+$ IEL from self $Ag^+$ and self $Ag^-$ mice to determine whether the TCR and costimulatory signals could affect the differentiation of the compromised less-responsive IEL. Activation requirements for IL-2 production (Fig. 3*A*) paralleled proliferative responses of $Tg^+$ IEL in primary and restimulated cultures. 2C IELs produced IL-2 in response to basal levels of p2Ca self Ag expressed by P815 cells, while increasing B7-mediated costimulation or the addition of exogenous peptide self Ag enhanced this response (IL-2 production: 377 (P815) compared with 502 (P815 + B7-1) and 1415 (P815 + p2Ca) pg/ml, respectively). In comparison, $Tg^+$ IELs from self $Ag^+$ mice made insignificant levels of IL-2 in response to P815 cells with or without B7-1 expression. Addition of p2Ca induced relatively low levels of IL-2, which were enhanced with expression of B7-1 by P815 cells (IL-2 production: 165 (P815 + p2Ca) vs 902 (P815/B7-1 + p2Ca)). IFN-γ production was not detected for self $Ag^+$ mice, suggesting that proliferation correlated with IL-2 production. Results of IFN-γ production correlated with IL-2 levels

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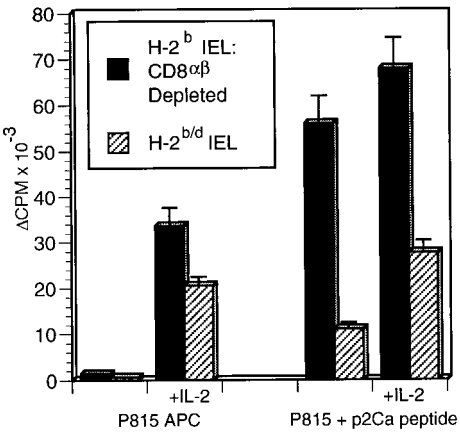


**FIGURE 4.** Differential activation threshold for prestimulated H-2$^b$ and H-2$^{b/d}$ IELs. Transgenic IELs from H-2$^b$ (solid) and H-2$^{b/d}$ mice (hatched) were prestimulated with P815 + p2Ca peptide + 20 U/ml IL-2. After 7 to 10 days in culture, cells were harvested and stimulated. All measurements were performed in triplicate and the data expressed as the mean, with an SE < 15%. Data shown are representative of three experiments.

for self Ag$^-$ mice, suggesting that the activation threshold was lower for Tg$^+$ IELs from self Ag$^-$ compared with self Ag$^+$ mice, and that signal 2 effects were detectable only after the threshold for signal 1 was met.

*Activation of prestimulated IEL populations*

It remained possible that the T cell unresponsiveness observed in self Ag$^+$ mice was due to chronic suppression in vivo by other cells or by chronic exposure to low levels of self Ag. To address this concern, cells were activated under optimal conditions and then retested after resting in vitro in the absence of self Ag-bearing APC. P815 cells were lysed in initial cultures by IELs, as confirmed by flow cytometric analysis as well as lack of response of IELs to p2Ca peptide in secondary culture without added APC (data not shown). To examine whether prestimulation of IELs altered the activation requirements of Tg$^+$ IEL from self Ag$^-$ and self Ag$^+$ mice, proliferative responses were assessed 7 days after stimulation in vitro with P815 plus B7-1, p2Ca, and IL-2. Upon restimulation in vitro, both self Ag$^-$ and self Ag$^+$ IEL responded at relatively low levels to P815 cells (Fig. 4) and B7-transfected P815 cells (data not shown). Proliferative responses of Tg$^+$ IEL from both strains were enhanced with addition of IL-2. Addition of p2Ca induced fivefold greater proliferative responses for Tg$^+$ IEL from self Ag$^-$ compared with self Ag$^+$ mice (stimulation index = 80 vs 15, respectively). Proliferative responses for Tg$^+$ IEL from self Ag$^-$ mice were also greater than IEL from self Ag$^+$ mice when T cells were cultured with p2Ca plus B7-1 transfectants even in the presence of IL-2 (data not shown). Thus, the proliferative defect in the T cells isolated from the self Ag$^+$ mice could not be restored by activation and expansion in vitro.

In restimulated cultures Tg$^+$ IELs from self Ag$^-$ but not self Ag$^+$ mice made IL-2 in response to P815 cells (Fig. 5). Addition of B7-1 expression increased IL-2 production for Tg$^+$ IEL from self Ag$^-$ mice by 73% (IL-2 for P815/B7 compared with P815: 455 and 262 pg/ml respectively). However, no effect of B7-1 was detected for prestimulated Tg$^+$ IELs from self Ag$^+$ mice cultured with P815 transfectants without p2Ca. Interestingly, expression of B7-mediated costimulation by P815 cells enhanced cytokine production for IELs from self Ag$^+$ mice when activated with p2Ca peptide but otherwise had little or no effect on other IELs tested. Taken together these data suggested that the increased requirement

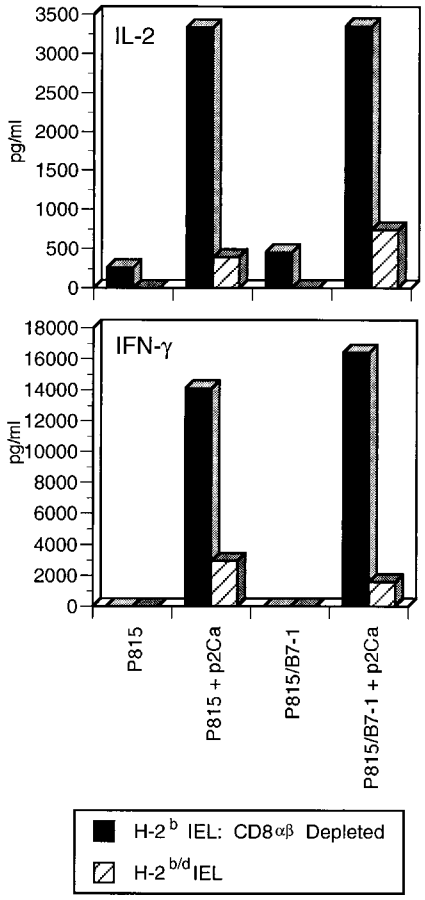


**FIGURE 5.** Cytokine response of preactivated IEL populations. IELs were activated under optimal conditions and then retested for cytokine production after resting in vitro in the absence of self Ag-bearing APC. Culture supernatant was collected at 48 h for the conditions indicated, and levels of IL-2 and IFN-γ measured by ELISA.

for signal 1 levels exhibited by fresh IEL from self Ag$^+$ mice was not reversible.

*Cytolytic response of hyporesponsive IEL populations*

To assess whether exposure to self Ag altered IEL cytolytic responses, Tg$^+$ IELs from self Ag$^+$ mice were compared with self Ag$^-$ mice. Tg$^+$ IEL from self Ag$^-$ mice lysed P815 cells without added peptide; however, Tg$^+$ IEL from self Ag$^+$ mice required added p2Ca to lyse P815 targets (Fig. 6, *A* and *B*). Interestingly, both the titration curve and the maximal lytic activity were greater for cells from self Ag$^+$ compared with self Ag$^-$ mice (percent specific lysis: 42% vs 22%, respectively).

Prestimulated IELs exhibited a similar pattern of cytolytic responses. Both self Ag$^-$ and self Ag$^+$ IELs lysed P815 targets when exogenous p2Ca peptide was added, but only self Ag$^-$ IELs lysed P815 cells expressing endogenous levels of self Ag (Fig. 6, *C* and *D*). These data suggested that a similarly increased threshold for T cell activation existed for induction of proliferation, cytokine production, and cytolysis for Tg$^+$ IEL in self Ag$^+$ mice.

## Discussion

The goal of this study was to assess the activation status and signaling capability of the residual IELs present in Ag-bearing TCR Tg mice. The results demonstrated that indeed the hyporesponsive IEL from Ag-bearing mice were defective in Ag-mediated stimulation. However, increases in TCR-mediated signal transduction

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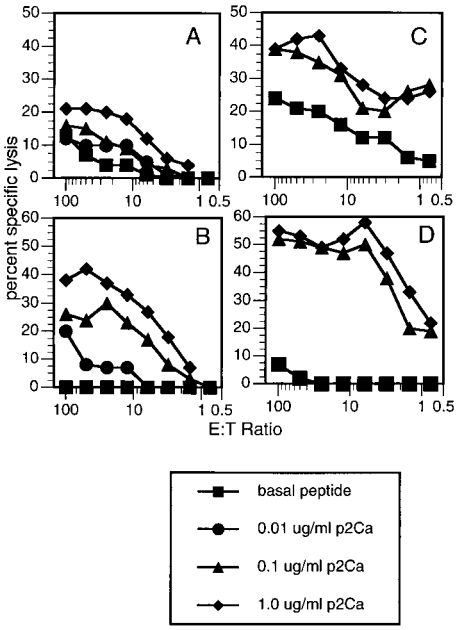


**FIGURE 6.** Enhanced signal 1 requirement for CTL response of IEL populations from self $Ag^+$ mice. Cytolytic responses of freshly isolated (*A*, *B*) and prestimulated (*C*, *D*) $Tg^+$ IEL from H-$2^b$ (*A*, *C*) and H-$2^{b/d}$ (*B*, *D*) mice are shown against $^{51}Cr$-labeled P815 targets ± p2Ca peptide. Effector:target ratios are indicated, and results converted to percent specific lysis of target cells. Data shown are representative of three experiments.

via the addition of exogenous antigenic peptide could, at least, partially correct the defect. Although IEL responses to self Ag were enhanced by the addition of B7-1-mediated costimulation (signal 2), signaling was unable to correct the functional defect of the hyporesponsive IEL isolated from the Ag-bearing animals. Rather, H-$2^{b/d}$ IEL responses, detected after signal 1 levels were supplemented with added p2Ca peptide, could be enhanced by expressing B7-1 on the P815 stimulator cells. Together, these results suggested that the defect in H-$2^{b/d}$ IEL responses was primarily related to an increased threshold for signal 1 but not signal 2.

Interestingly, chronic exposure to self Ags in vivo induced a functional defect in H-$2^{b/d}$ IELs similar to that induced by stimulation with partial TCR agonists, such as altered peptide ligands (APLs) or soluble anti-CD3 mAbs. Previous studies have shown that stimulation of T cells by partial agonists induces cytolytic function and some cytokine production (e.g., IL-4 or IFN-γ) without proliferation or IL-2 production (18–23). This pattern of response parallels results of $Tg^+$ IEL from self $Ag^+$ mice where cytolytic response, IL-4, and IFN-γ production were retained but IL-2 production and proliferation were down-regulated compared with $Tg^+$ IEL responses in self $Ag^-$ mice. In vitro stimulation of T cells with APLs suggests that partial agonists provide a qualitatively distinct signal to T cells. Partial agonist stimulation of T cells leads to partial phosphorylation of the TCR-ζ chains and fails to fully activate ZAP-70 tyrosine kinase (22, 24) or induce a sustained intracellular $Ca^{2+}$ flux (25). Based on the findings of the present in vivo model, we speculate that signaling in $Tg^+$ IELs in self $Ag^+$ mice may be similarly altered, resulting in the requirement for elevated levels of signal 1 to overcome the hyporesponsive state created by chronic exposure to self Ag.

It is possible that disorders such as inflammatory bowel disease are begun with events that increase TCR-mediated signaling. These results suggested that autoimmunity in the intestine may be initiated by elevated levels of TCR signaling that overcome partial agonist effects induced by self Ag. For example, increased expression and peptide loading of class I MHC molecules with high affinity immunogenic Ag (self Ag or Ag mimics) may be enhanced in the setting of viral infections (26–28). Likewise, superantigens may induce TCR signaling that exceeds activation requirements. Activation of self-reactive IELs may induce cytolytic responses against epithelial cells. Disruption of the mucosal barrier may lead to widespread activation of mucosal immune cells with proinflammatory enteric Ags. Once activation of self-reactive T cells occurs, the extent of tissue inflammation may depend on persistent expression of immunogenic TCR agonists and signal 2-mediated costimulation. In this setting, increased costimulation expressed by APC may not be sufficient to initiate responses but may enhance IEL expansion, survival, and cytokine production induced by TCR signaling. Thus, these data suggest that increased levels of immunogenic TCR agonists (high levels of self Ag, high affinity Ag, mimics, superantigens, etc.) initiate autoreactivity in the intestine, whereas enhanced costimulation potentiates the severity of tissue damage.

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## References

1. Poussier, P., P. Edouard, C. Lee, M. Binnie, and M. Julius. 1992. Thymus-independent development and negative selection of T cells expressing T cell receptor alpha/beta in the intestinal epithelium: evidence for distinct circulation patterns of gut- and thymus-derived T lymphocytes. *J. Exp. Med. 176:187.*
2. Rocha, B., P. Vassalli, and D. Guy-Grand. 1991. The V beta repertoire of mouse gut homodimeric alpha $CD8^+$ intraepithelial T cell receptor alpha/$beta^+$ lymphocytes reveals a major extrathymic pathway of T cell differentiation. *J. Exp. Med. 173:483.*
3. Poussier, P., H. S. Teh, and M. Julius. 1993. Thymus-independent positive and negative selection of T cells expressing a major histocompatibility complex class I restricted transgenic T cell receptor alpha/beta in the intestinal epithelium. *J. Exp. Med. 178:1947.*
4. Lefrancois, L., and L. Puddington. 1995. Extrathymic intestinal T-cell development: virtual reality? *Immunol. Today 16:16.*
5. Lefrancois, L., and S. Olson. 1994. A novel pathway of thymus-directed T lymphocyte maturation. *J. Immunol. 153:987.*
6. Lin, T., G. Matsuzaki, H. Yoshida, N. Kobayashi, H. Kenai, K. Omoto, and K. Nomoto 1994. $CD3^-CD8^+$ intestinal intraepithelial lymphocytes (IEL) and the extrathymic development of IEL. *Eur. J. Immunol. 24:1080.*
7. Guehler, S. R., J. A. Bluestone, and T. A. Barrett. 1996. Immune deviation of 2C transgenic intraepithelial lymphocytes in antigen-bearing hosts. *J. Exp. Med. 184: 493.*
8. Barrett, T. A., T. F. Gajewski, D. Danielpour, E. B. Chang, K. W. Beagley, and J. A. Bluestone. 1992. Differential function of intestinal intraepithelial lymphocyte subsets. *J. Immunol. 149:1124.*
9. Kranz, D., D. H. Sherman, M. V. Sitkovsky, M. S. Pasternack, and H. N. Eisen. 1995. Immunoprecipitation of cell surface structures of cloned cytotoxic T lymphocytes by clone-specific antisera. *Proc. Natl. Acad. Sci. USA 81:573.*
10. Ledbetter, J. A., R. Rouse, H. Micklem, and L. A. Herzenberg. 1980. T cell subsets defined by expression of Lyt-1,2,3 and Thy-1 antigens: two-parameter immunofluorescence and cytotoxicity analysis with monoclonal antibodies modifies current views. *J. Exp. Med. 152:280.*
11. Ledbetter, J. A., and L. A. Herzenberg. 1979. Xenogenic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev. 47:63.*
12. Nelson, B. H., J. D. Lord, and P. D. Greenberg. 1994. Cytoplasmic domains of the interleukin-2 receptor beta and gamma chains mediate the signal for T-cell proliferation. *Nature 369:333.*
13. Gunning, P., J. Leavitt, G. Muscat, S. Y. Ng, and L. Kedes. 1987. A human beta-actin expression vector system directs high-level accumulation of antisense transcripts. *Proc. Natl. Acad. Sci. USA 84:4831.*
14. Udaka, K., T. J. Tsomides, P. Walden, N. Fukusen, and H. N. Eisen. 1993. A ubiquitous protein is the source of naturally occurring peptides that are recognized by a $CD8^+$ T-cell clone. *Proc. Natl. Acad. Sci. USA 90:11272.*
15. Udaka, K., T. J. Tsomides, and H. N. Eisen. 1992. A naturally occurring peptide recognized by alloreactive $CD8^+$ cytotoxic T lymphocytes in association with a class I MHC protein. *Cell 69:989.*
16. Lefrancois, L., and T. Goodman. 1989. In vivo modulation of cytolytic activity and Thy-1 expression in TCR-gamma $delta^+$ intraepithelial lymphocytes. *Science 243:1716.*

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17. Sperling, A. I., J. A. Auger, B. D. Ehst, I. C. Rulifson, C. B. Thompson, and J. A. Bluestone. 1996. CD28/B7 interactions deliver a unique signal to naive T cells that regulates cell survival but not early proliferation. *J. Immunol. 157:3909.*
18. Evavold, B. D., and P. M. Allen. 1991. Separation of IL-4 production from Th cell proliferation by an altered T cell receptor ligand. *Science 252:1308.*
19. Evavold, B. D., J. Sloan-Lancaster, and P. M. Allen. 1993. Tickling the TCR: selective T-cell functions stimulated by altered peptide ligands. *Immunol. Today 14:602.*
20. Sloan-Lancaster, J., B. D. Evavold, and P. M. Allen. 1993. Induction of T-cell anergy by altered T-cell-receptor ligand on live antigen-presenting cells. *Nature 363:156.*
21. Evavold, B. D., J. Sloan-Lancaster, B. L. Hsu, and P. M. Allen. 1993. Separation of T helper 1 clone cytolysis from proliferation and lymphokine production using analog peptides. *J. Immunol. 150:3131.*
22. Madrenas, J., R. L. Wange, J. L. Wang, N. Isakov, L. E. Samelson, and R. N. Germain. 1995. Zeta phosphorylation without ZAP-70 activation induced by TCR antagonists or partial agonists. *Science 267:515.*
23. Evavold, B. D., S. G. Williams, B. L. Hsu, S. Buus, and P. M. Allen. 1992. Complete dissection of the Hb(64-76) determinant using T helper 1, T helper 2 clones, and T cell hybridomas. *J. Immunol. 148:347.*
24. Sloan-Lancaster, J., A. S. Shaw, J. B. Rothbard, and P. M. Allen. 1994. Partial T cell signaling: altered phospho-zeta and lack of zap70 recruitment in APL-induced T cell anergy. *Cell 79:913.*
25. Wulfing, C., J. D. Rabinowitz, C. Beeson, M. D. Sjaastad, H. M. McConnell, and M. M. Davis. 1997. Kinetics and extent of T cell activation as measured with the calcium signal. *J. Exp. Med. 185:1815.*
26. Tanaka, K. 1994. Role of proteasomes modified by interferon-gamma in antigen processing. *J. Leukocyte Biol. 56:571.*
27. Tovey, M. G., E. Benizri, J. Gugenheim, G. Bernard, P. Eid, and B. Blanchard. 1996. Role of the type I interferons in allograft rejection. *J. Leukocyte Biol. 59:512.*
28. Pestka, S. 1994. Identification of functional type I and type II interferon receptors. Hokkaido J. Med. Sci. 69:1301.

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