Changes in the strength of co-stimulation through the B7/CD28 pathway alter functional T cell responses to altered peptide ligands

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Abstract

T cells require a TCR and a co-stimulatory signal for activation. We have examined the effect of the strength of TCR and co-stimulatory signals on proliferation and production of cytokines by differentiated T cell clones. The TCR signal was varied using antigen dose and altered peptide ligands. The co-stimulatory signal was varied by using as antigen-presenting cells, Chinese hamster ovary cell transfectants that express different levels of the B7-1 molecule with similar levels of MHC class II. Our results show that the level of co-stimulation has a profound effect on the response to an antigen, and that a strong co-stimulatory signal can convert a weak agonist into a full agonist and an agonist into a superagonist. Antigenicity is not absolute but a function of the strengths of the TCR and co-stimulatory signals. Increasing the strength of co-stimulation can lower antigen concentration required for maximal proliferative responses by T cell clones by 5 log. These results show that the level of expression of co-stimulatory molecules will profoundly regulate T cell clonal expansion and effector functions.

Introduction

Studying the factors that influence CD4\(^+\) T cell activation and differentiation is critical to our understanding of the role of these cells in health and disease. The activation of T cells requires at least two signals, an antigenic signal (signal 1) and a co-stimulatory signal (signal 2) delivered by antigen-presenting cells (APC) (1). Antigenic signal alone in the absence of co-stimulation can induce antigen specific unresponsiveness (anergy) (2–5).

A number of groups have attempted to study the parameters that influence T cell activation as well as differentiation. One such approach has been to vary the TCR signal (signal 1) either by altering the antigen concentration (6–8) or by using altered peptide ligands (APL) (9–13). Altering the TCR signal shows that high antigen dose or high-affinity ligand can induce T_{h}1 cells that secrete IFN-\(\gamma\) and IL-2, whereas low antigen dose or low-affinity ligand can induce T_{h}2 cells that produce IL-4. We have recently identified an APL that delivers a more potent signal 1 to T cells than the cognate ligand. This APL hyperstimulates T cells in that it induces strong proliferative responses at low antigen dose and also induces secretion of cytokines [IL-2 and tumor necrosis factor (TNF)-\(\alpha\)] not observed with cognate ligand (13). Based on these and other studies a concept of ‘strength of signal 1’ has evolved and has been postulated to play a crucial role in T cell differentiation. However, it is not clear how changes in signal 2 (co-stimulation) will integrate with the strength of signal 1 to affect activation thresholds and effector functions of T lymphocytes.

In this study, we have addressed the role of the strength of co-stimulation (signal 2) in functional responses of T cell clones to cognate and APL. Using as APC, Chinese hamster ovary (CHO) cells that express different levels of B7-1 and a similar level of MHC class II molecules, we show that the level of co-stimulation has a dramatic effect on (i) the antigen
dose required for maximal proliferative responses of T cell clones, (ii) the secretion of different cytokines and (iii) conversion of a weak/partial agonist to a full agonist.

Methods

Antigens

Proteolipid protein (PLP) (139–151) HSLGKWLGHPDKF (W144) peptide, or its altered forms PLP (A144) HSLGKKLGHPDKF, PLP (Q144) HSLGQLGHPDKF and PLP (A147) HSLGKWLGAPDKF, were synthesized in the laboratory of Dr R. Laursen (Boston University Boston, MA) using FMOC chemistry on a Milligen synthesizer (model 9050; Perspective Biosystems, Framingham, MA).

Antibodies

10.2.16, a mouse anti I-Aα mAb that cross-reacts with I-Aβ, was used as the primary antibody for monitoring MHC class II expression on CHO cell transfectants. For detection of B7-1 expression, either FITC-conjugated human CTLA-4-Ig or 1G10, a rat anti-mouse B7-1 antibody, was used. For detection of MHC class II by indirect immunofluorescence either FITC- (Caltag, San Francisco, CA) or phycoerythrin- (Southern Biotechnology Associates, Birmingham, AL) conjugated goat anti-mouse secondary antibody was used. FITC-conjugated goat anti-rat IgG (Caltag) was used as the secondary antibody whenever 1G10 or GL1 was used as a primary antibody for detection of B7-1 or B7-2 expression respectively. When FITC-conjugated CTLA-4-Ig (5) was used for staining cells, negative controls included cells stained with isotype matched FITC-conjugated Ig. Results were analyzed on a Coulter flow cytometer (Epics XL-MCL).

Generation of stable transfectants

Full-length I-Aα and β cDNA clones in Bluescript vector were a gift of Dr S. Miller (Northwestern University, Evanston, IL). The plasmids were digested with EcoRI, and the I-Aα and β chains were isolated, and cloned into an expression vector, SRα (14). Linearized plasmids (25 µg) containing I-Aα and β chains respectively, along with 5 µg of linearized SV2-Neo-SP65 plasmid were transfected by electroporation into CHO cells, a MHC class II-negative, B7-negative cell line. After selection in medium containing G418 at 400 µg/ml, cells were sorted on a Coulter cell sorter (Epics Elite ESP) for MHC class II expression by indirect immunofluorescence, using 10.2.16 mAb. The sorted cells were >95% positive. The cells were cultured for 4 weeks, resorted and cloned by limiting dilution (0.5 cells/well). Transfectants expressing MHC class II were monitored for stable expression over a period of 4 weeks. One stable transfectant was selected for further transfection. Linearized murine B7-1 plasmid (50 µg) (15) along with 5 µg of linearized pPGK-hygro vector containing the hygromycin-resistance gene were transfected by electroporation into the CHO cell line stably expressing MHC class II. The transfected cells were selected in selection media containing 400 µg/ml G418 and hygromycin. The antibiotic-resistant cells were sorted for MHC class II and B7 expression using 10.2.16 and FITC-conjugated CTLA-4-Ig. Double-positive cells were cloned by limiting dilution (0.5 cells/well), and were screened for B7-1 and MHC class II expression using 1G10 and 10.2.16 mAb. Clones expressing different levels of B7-1 with similar levels of MHC class II were selected for this study. Cell phenotypes were re-examined before each experiment. All the transfectants were also phenotyped with anti-B7-2 mAb GL1 and found to be negative.

T cell clones

The generation of PLP (139–151)-specific T cell clones ID9 and IE6 has been described elsewhere (16). In brief, ID9 was generated from lymph nodes of SJL mice after immunization with PLP peptide (139–151) in complete Freund's adjuvant (CFA; Difco, Detroit, MI). The lymph node cells were obtained 7–10 days after immunization, stimulated with W144 peptide and propagated in DMEM containing 0.6% T cell growth factor (T-STEM; Collaborative Biomedical Research, Bedford, MA) and 0.06% recombinant mouse IL-2. IE6 was generated by immunizing SJL mice with peptide W144, followed by i.p. administration of monoclonal anti-B7-1 antibody. This clone was expanded and propagated as described above for ID9. Q1.1B6.6E3 (hereafter referred to as B6E) was generated by immunizing SJL mice with altered peptide Q144 in CFA (13). The clone was expanded and propagated in IL-2 as described earlier (13).

In vitro proliferation assays

CHO cell transfectants were treated with 50 µg/ml mitomycin C (Sigma, St Louis, MO) for 21 h at 37°C. The cells were harvested with 10 mM EDTA in PBS, washed extensively and incubated on ice for ~30 min in media. The cells were washed again 3 times and counted. T cell clones were washed 3 times in DMEM and counted. Mitomycin C-treated CHO cell transfectants (105 cells/well) or irradiated (5000 rad) spleen cells (5×106 cells/well) and ID9, IE6 or B6E T cell clones (103–104 cells/well) were cultured in triplicate in 96-well flat-bottom plates (Becton Dickinson, Lincoln Park, NJ) in the presence of antigen, for 48 h. [3H]Thymidine (1 µCi/well) was added for the last 16 h before harvesting the cells. The [3H]thymidine incorporation was determined in a scintillation counter (model LS 5000; Beckman Instruments) and the result was expressed as mean c.p.m. Supernatants were harvested from the cultures after 40 h for determining the type and amount of cytokines produced. Supernatants were stored at −80°C until assayed.

Cytokine ELISA

Culture supernatants were diluted 1:2 and tested for the presence of cytokines by ELISA as described (16). IL-2, IL-4, IL-10 and TNF-α ELISA components (antibodies and cytokine standards) were obtained from PharMingen (San Diego, CA). In brief, ELISA plates (Immulon 4; Dynatech, Chantilly, Virginia) were coated with purified rat mAb to mouse IL-2 (clone JES6-1A12), IL-4 (clone BVD-1D11), IL-10 (clone JES5-2A5) and TNF-α (clone MP6-XT22). Reombinant mouse cytokines IL-2, IL-4, IL-10 and TNF-α were used to construct standard curves. Biotinylated rat mAb to mouse IL-2 (clone JES6-5H4), IL-4 (BVD6-24G2) and IL-10 (SXC-1), and biotinylated polyclonal rabbit anti-mouse TNF-α were used as secondary detecting antibodies. IFN-γ components were obtained
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Fig. 1. Expression of I-A\(^d\) and B7-1 in CHO cell transfectants. CHO cells were transfected with I-A\(^d\) and mouse B7-1 as described in Methods. Controls also included a transfectant that expressed only I-A\(^d\). The expression of MHC class II and B7-1 was determined by 10.2.16 (I-A\(^d\)) and 1G10 (B7-1) mAb by indirect immunofluorescence. Negative control included cells stained with anti-B7-2 antibody (GL1) which were found to be identical to cells stained with the secondary antibody alone. MFI is indicated in the upper right of each histogram and the clone description is included on the right.

Varying the level of B7-1 expression has a dramatic effect on the antigen dose required for maximal functional responses by T cell clones

Table 1 shows the antigen specificity and cytokine profile of the T cell clones used in this study. The cytokine profiles of these clones were obtained with cognate ligand using irradiated syngeneic spleen cells as APC. APL that act as partial or full agonists for each of the T cell clones are also given in Table 1. To study the interplay of strength of signal 1 with signal 2, we investigated whether differences in strength of co-stimulation could lead to different functional responses with increasing TCR signal. A panel of transfectants expressing different levels of B7-1 with similar levels of MHC class II were used as APC to stimulate a T cell clone, IE6, with its cognate antigen W144. Control APC included irradiated spleen cells or CHO/I-A\(^d\) that expressed MHC class II alone. The response was studied over a 7 log range of antigen dose (0.0001–100 \(\mu\)g/ml). Our results demonstrate that the level of co-stimulation had a profound effect on the proliferative response as well as cytokine production by this clone (Fig. 2). The transfectant expressing the highest level of B7-1 [CHO/I-A\(^d\)/B7-1 (c8)] shifted the antigen dose–response curve 4 log compared to transfectants that expressed the lowest level of B7-1 [CHO/I-A\(^d\)/B7-1 (c2)] or the irradiated syngeneic spleen cells. The proliferative response increased in the order of B7-1 expression, c8 > c8.1 > c11 > c2, and was proportional to B7-1 expression levels. This pattern was also reflected in cytokine production by clone IE6. As the co-stimulatory signal increased, both IFN-\(\gamma\) and IL-10 production required a lower antigen dose. In the absence of co-stimulation, IL-4 was detectable only at the highest antigen dose. IL-4 production was augmented by B7-1 co-stimulation; however, IL-4 production was much less dependent on the level of co-stimulation. Also, IL-4 required a lower antigen dose for maximal production compared to IFN-\(\gamma\) or IL-10 (Fig. 2). The cognate antigen W144 did not induce detectable IL-2 production from the IE6 T cell clone (data not shown). For this clone at the highest antigen doses, irradiated spleen cells consistently induced higher levels of cytokines, particularly IL-4, than the transfectants, probably due to expression of additional accessory molecules on the APC besides B7 (Fig. 2). Thus, these experiments showed that varying the strength

from Genzyme (Cambridge, MA). Monoclonal hamster anti-mouse IFN-\(\gamma\) was used as the primary antibody, polyclonal goat anti-mouse IFN-\(\gamma\) was used as secondary antibody. Assays were developed in TMB Microwell peroxidase substrate (Kirkegaard & Perry, Gaithersberg, MD) and read at 450 nm after addition of stop solution using a model 3550 Microplate Reader (BioRad, Hercules, CA). Each clone was assayed for all five cytokines and if the data is not presented, this indicates the cytokine was below the level of detection.

Results

Generation of CHO cell transfectants expressing I-A\(^d\) and different levels of B7-1

A series of CHO cell transfectants expressing similar levels of I-A\(^d\) and varying levels of B7-1 were used as APC in this study (Fig. 1). CHO/I-A\(^d\)/B7-1 (clones 2, 11, 8.1 and 8) expressed increasing levels of B7-1, with mean fluorescence intensities (MFI) of 160, 290, 470, and 640 respectively. As control, a transfectant (CHO/I-A\(^d\)) that expressed a similar level of MHC class II but no B7 was used. The transfectants were also phenotyped with anti-B7-2 antibody (GL1) and, as expected, all were negative (Fig. 1).

Table 1. Antigenic specificity and cytokine profiles of T cell clones used in this study

<table>
<thead>
<tr>
<th>T cell clone</th>
<th>Cognate ligand</th>
<th>Cytokine profile</th>
<th>Agonist</th>
<th>Weak agonist</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1.1B6.IE3</td>
<td>Q144 (HSLGKQLGPDKK)</td>
<td>IFN-(\gamma)</td>
<td>None</td>
<td>A144</td>
</tr>
<tr>
<td>IE6</td>
<td>W144 (HSLGKWLPDKK)</td>
<td>IFN-(\gamma), IL-4, IL-10</td>
<td>A147</td>
<td>None</td>
</tr>
<tr>
<td>ID9</td>
<td>W144 (HSLGKWLPDKK)</td>
<td>IFN-(\gamma), IL-2, IL-10</td>
<td>None</td>
<td>A147</td>
</tr>
</tbody>
</table>

The antigen specificities and the cytokine profiles of the T cell clones were defined using cognate ligand and irradiated syngeneic spleen cells as APC. The sequences of the cognate ligands, including the native form of PLP (139–151), W144, and the altered form, Q144, are indicated in the parentheses.
Fig. 2. Proliferative response and cytokine profile of T\textsubscript{H}0 clone IE6 using the cognate ligand W144 and a panel of APC. APC included mitomycin C-treated CHO cells expressing similar levels of I-A\textsuperscript{b} and varying levels of B7-1. A CHO cell transfectant that expressed I-A\textsuperscript{b} alone and irradiated spleen cells were also used as controls. The functional response was assessed over a wide range of antigen dose (0.0001–100 µg/ml). Proliferation was measured by \textsuperscript{3}H\textsuperscript{}thymidine incorporation. Cytokine analysis was done on 40 h culture supernatants by ELISA. The data is representative of two independent experiments.
of co-stimulation had a dramatic effect on shifting the antigen dose required for maximal functional responses of T cell clones.

Higher levels of co-stimulation and agonist ligands hyper-stimulate T cell clones and induce production of additional cytokines

To further confirm whether the effects of strength of co-stimulation on functional responses of T cell clones was generalizable, we studied two additional T cell clones with different antigen specificities (Table 1). The strength of co-stimulation was varied as before by varying the level of B7-1 expression. Two transfectants CHO/I-A\*β7-1 (c11) and CHO/I-A\*β7-1 (c8) that expressed low and high levels of B7-1 (MFI of 290 and 640 respectively, see Fig. 1 for details) were used as APC. Control APC also included irradiated spleen cells and CHO/I-A\* that expressed MHC class II alone. The T cell clone 1B6, was stimulated with its cognate ligand Q144 using this panel of APC. The high expressor of B7-1 [CHO/I-A\*β7-1 (c8)] was able to induce a much stronger proliferative response and shifted the antigen dose–response curve 3 log compared to the low B7-1 expressor, CHO/I-A\*β7-1 (c11), irradiated spleen cells or CHO/I-A\* (Fig. 3a). As before, higher co-stimulation induced higher levels of IFN-γ production at low antigen dose. The clone 1B6 does not produce IL-2 when activated with cognate ligand Q144 and splenic APC even at the maximal antigen concentration tested (13). However, when this clone was activated with cognate ligand and high expressor B7-1 CHO transfectant, there was induction of IL-2. We have observed similar induction of IL-2 from 1B6 transfected clone when activated with superagonist ligand L144 (13). Thus high B7-1 expressor CHO cells not only induce heteroclitic proliferative responses and increased production of cytokines when activated with the cognate ligand, they induce production of new cytokines (e.g., IL-2 from 1B6) that are not induced by the cognate ligand with irradiated splenic APC. Thus, these data suggest that increased co-stimulation through B7-1 can change an agonist into a superagonist.

Strength of co-stimulation also had a dramatic effect on the functional response of T cell clone ID9, for which W144 is the cognate ligand. As observed with clone 1B6, when clone ID9 was stimulated with its cognate ligand (W144), antigen presentation by transfectants expressing high levels of B7-1 shifted the antigen dose–response curve 3 log compared to the low B7-1 expressor or irradiated spleen cells (Fig. 3b). Moreover, IL-2 was produced only with higher co-stimulation and antigen dose, and was found to be completely dependent on co-stimulation as it was not produced when CHO/I-A\* or irradiated spleen cells were used as APC (Fig. 3b). Thus the strength of the T cell response is consistently dependent on the strength of the co-stimulatory signal.

Using cognate ligand (agonist) or altered forms of this ligand, we have identified APL that have similar binding affinity for I-A\* but either hypostimulate or hyperstimulate T cell clones (Table 1). These APL provide unique tools that can be used to study the effects of varying the TCR signal on the functional response of T cell clones without affecting MHC loading. The T cell clone, IE6, was stimulated with an APL, A147, using the same panel of APC as before. A147 induces a slightly stronger (heteroclitic) T cell response compared to the cognate ligand (W144) with ~1 log shift in the antigen dose–response curve using splenic APC but this ligand induces qualitatively the same cytokines from this T cell clone (compare Figs 2 and 3c). When clone IE6 was stimulated with APL A147, antigen presentation by transfectants expressing high levels of B7-1 shifted the antigen dose–response curve for proliferation and cytokine production >2 log compared to the low B7-1 expressor or 4 log compared to irradiated spleen cells (Fig. 3c). With strong B7-1 co-stimulation, proliferation was still maximal even at the lowest dose of antigen, 0.0001 µg/ml. T cells and APC without antigen did not induce proliferative responses or cytokine production (data not shown). IL-2, which is not produced by clone IE6 in response to W144, is produced in response to A147 but production required higher strengths of co-stimulation as well as high antigen dose (Fig. 3c). Interestingly, again IL-4 production was least affected by differences in co-stimulation and while co-stimulation augmented IL-4 production, at high antigen dose this T cell clone produced IL-4 without co-stimulation. At the highest antigen dose, irradiated spleen cells were able to induce higher amounts of IL-4 than the transfecants (Fig. 3c). Neither IE6 or ID9 produced detectable TNF-α. Thus, these results show that a high level of co-stimulation can dramatically reduce the antigen dose required for maximal proliferative responses and cytokine production, essentially converting a strong agonist into a superagonist. Moreover, these results also show that IL-4, IL-10, IFN-γ and IL-2 have different co-stimulatory thresholds for production. With increased co-stimulation, there is a qualitatively different cytokine profile, i.e. induction of additional cytokines such as IL-2 that are not seen either with spleen cells or CHO cells that deliver weaker co-stimulatory signals.

High strength of co-stimulation can convert a weak/partial agonist into a full agonist

Since strong co-stimulation could convert a strong agonist into a superagonist, we examined the effect of strong co-stimulation on activation by a weak/partial agonist. We have generated a panel of partial/weak agonists by single amino acid substitution of the cognate ligand (Table 1). A144 is an APL which is a weak agonist for T cell clone 1B6, based on its ability to induce low functional responses compared to the cognate antigen (Q144) using splenic APC (cf. Figs 3a and 4a). With splenic APC, the weak agonist A144 can stimulate low level IFN-γ production but no proliferation (Fig. 4a). Interesting differences were observed with stronger co-stimulation. Using the weak agonist A144, proliferation was not observed with CHO cells expressing lower levels of B7-1, MHC class II alone or irradiated spleen cells but transfectants expressing the highest levels of B7-1 were able to stimulate proliferation at high antigen dose (Fig. 4a). Moreover, the proliferative response with high co-stimulation was equivalent to that obtained with the cognate ligand Q144 using splenic APC (cf. Figs 3a and 4a). Transfectants expressing the highest level of B7-1 shifted the antigen dose–response curve for IFN-γ production 1 log compared to splenic APC. None of the APC could stimulate IL-2 production by 1B6 in response to A144, suggesting that IL-2 production requires high TCR signal as well as co-stimulation for production. Thus, with high strength of co-stimulation altering T cell response 411
co-stimulation a weak agonist response could be converted to a full agonist response.

To further confirm that strong co-stimulation can convert a weak agonist into a full agonist, we examined the response of another T cell clone ID9. A147 is an APL which is a weak agonist for T cell clone ID9, based on its ability to induce low functional responses compared to the cognate antigen (W144) using splenic APC (cf. Figs 3b and 4b). Clone ID9 was stimulated with A147 using the same panel of APC to study the functional outcome. The highest B7-1 expressor, CHO/I-A^d/B7-1 (c8), was found to be the most effective APC for stimulating the proliferative response as well as cytokine production and at the highest antigen dose the response was equivalent to that observed with the cognate ligand using irradiated spleen cells as APC (cf. Figs 3b and 4b). IFN-γ was produced only with high co-stimulation as well as high antigen dose, whereas IL-10 could be produced by other APC providing weaker co-stimulation; however, high co-stimulation induced maximal IL-10 production. This suggests that in this clone, higher strengths of signal 1 and 2 are required for IFN-γ than for IL-10 production. IL-2 was not detectable when clone ID9 was stimulated by A147, suggesting once again...
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that a strong TCR signal as well as co-stimulation are essential for IL-2 secretion (Fig. 4b).

Thus, varying the strength of co-stimulation produced a dramatic reduction (2–5 log) in the antigen dose required for proliferation and cytokine production. Higher co-stimulation could convert a weak agonist to a full agonist and induce additional cytokines not observed with the cognate ligand and splenic APC.

Discussion

In this study we have examined the effect of the strength of TCR and co-stimulatory signals on proliferation and production of cytokines by differentiated T cell clones. The TCR signal was varied using antigen dose and APL. The co-stimulatory signal was varied by using as APC, CHO cell transfectants that express different levels of the B7-1 molecule with similar levels of MHC class II. We find that the strength of TCR and co-stimulatory signals determines the T cell response, and discuss the implications for the induction of an autoimmune or anti-tumor response.

The strength of co-stimulation had a profound effect on the antigen dose required for maximal proliferation. At a given strength of TCR signal, we show that proliferation was dependent on the level of co-stimulation. With higher levels of co-stimulation significantly lower antigen dose was required for induction of a strong proliferative response. High levels of co-stimulation could lower the required antigen dose up to 5 log. Our results are consistent with earlier studies, where co-stimulation could lower the antigen dose as well as the number of TCR molecules required for maximal proliferation by T cell clones (17). While a number of studies have shown the importance of co-stimulatory signals or of TCR signals, previous studies have not examined the effect of simultaneously varying TCR and co-stimulatory signals.

The capacity of the strength of co-stimulation to shift the antigen dose–response curve also applies to APL. The response to APL is generally defined using splenic APC. We find that strong co-stimulation can convert a weak/partial agonist into an agonist and convert an agonist into a superagonist. Presentation of a partial agonist with strong co-stimulation can lead to the production of additional cytokines.
not seen with splenic APC. As discussed below this result may have an important bearing on the induction of autoimmunity and anti-tumor immunity.

Finally, our study further reveals that each of the cytokines examined, i.e. IL-4, IL-10, IFN-γ and IL-2, has a different co-stimulatory threshold for production that varies depending on the strength of the TCR signal. In differentiated T cell clones, IL-4 production was induced by relatively low co-stimulation and antigen dose and while IL-4 production was augmented by co-stimulation, it was least affected by increases in the strength of co-stimulation. IL-10 and IFN-γ production require intermediate strengths of co-stimulation as well as TCR signal. In contrast, IL-2 production consistently required both higher co-stimulation as well as higher antigen dose (Fig. 3a-c). Thus, the T cell response is not simply on/off but is dictated by the strengths of TCR and co-stimulatory signals.

Previous studies have shown that the strength of the TCR signal dictates cytokine production by naive T cells. In these studies, low dose of antigen induced a Th2 phenotype, whereas high antigen dose shifted the naive T cells towards Th1 (6,7). Whether variation in co-stimulation would similarly affect T cell differentiation in naive T cells remains to be determined. Our data are consistent with a number of earlier studies that have altered the strength of the TCR signal by varying the antigen dose (6–8) or using APL (9–13) to show that the Tc1 cytokines, IFN-γ and IL-2, require either high antigen dose or high-affinity ligand for production. A recent study (8) showed that higher strength of TCR signal recruits more cells into the IFN-γ-producing pool, increases the amount of IFN-γ produced per cell and also elicits IL-2 production from these cells. This is consistent with our recent data showing that hyperstimulatory T cell ligands will induce IL-2 and TNF-α from an IL-4/IFN-γ-producing Tc1 clone (13). Also, studies where co-stimulation was blocked with CTLA-4–Ig showed a loss of IL-2 secretion, with IL-4 production being unaffected (18). Thus, our findings bring in another dimension and complexity in that we show an interplay between signals 1 and 2 in terms of T cell differentiation, and that the same outcome can be achieved if signal 2 is varied. In addition, we show that when the TCR signal is weak such as with a weak/partial agonist, the strength of co-stimulation then determines the functional outcome of the T cell response in terms of proliferation and cytokine production.

These findings have a direct bearing on the development of autoimmunity where high levels of co-stimulatory molecules might trigger autoreactive T cells. This is supported by studies where the expression of B7 and MHC class II molecules as transgenes on the islets resulted in autoimmune destruction of β cells in the pancreas (19). Furthermore, it has been shown that after activation with IFN-γ, astrocytes express B7 molecules and can prime naive antigen-specific T cells in vitro (20). This result suggests that an ongoing inflammatory response in a target organ can induce co-stimulatory molecules such as B7 on non-professional APC, making them capable of activating autoreactive T cells, which would then amplify the autoimmune T cell response and subsequently cause tissue damage by promoting a pro-inflammatory environment. Moreover, recent studies have shown tissue-specific up-regulation of B7-1 in EAE (21). The ability of high strength of co-stimulation to convert a weak agonist into an agonist may allow an infectious agent displaying a cross-reactive epitope (analogous to a weak agonist) to trigger an autoimmune response. If presented by an APC that overexpresses co-stimulatory molecules, the cross-reactive epitope may activate autoreactive T cells that would not respond with only moderate or weak co-stimulation.

In EAE, the involvement of Tc1 cells is well documented (22–24), whereas Tc2 cells mediate protection by antagonizing Tc1 cells (25–28). Moreover, adoptive transfer of T cells that are transfected with cDNAs for IL-4 or IL-10 as a transgene has been shown to confer protection (29,30). IL-4 has also been shown to be involved in T cell tolerance induced by aqueous protein antigens in vivo (31). This would suggest a possible mechanism by which Tc2 cells can regulate immune responses. Studies carried out in our laboratory have shown that APL can confer protection to EAE by either antagonizing Tc1 responses (32) or by stimulating a subpopulation of T cells that produces Tc2/Tc0 cytokines (10, 28). Moreover, blockade of the CD28/B7-1 pathway has been shown to prevent clinical relapses of murine EAE (33). Our present study shows that the strength of co-stimulation can influence the production of cytokines. Inflammatory cytokines such as IFN-γ require higher strengths of co-stimulation for their secretion. Furthermore, we also demonstrate that higher co-stimulation can convert a weak agonist into a full agonist. This data suggests that cross-reactive analogs of the autoantigen which normally do not stimulate autoreactive T cells may be able to affect T cell differentiation and with high levels of co-stimulation change a protective Tc2 response into a pathogenic Tc1 response. Thus lowering both the TCR and co-stimulatory signals may be necessary for inducing a Tc1 response.

Similarly, transfection of B7 into tumors has been shown by many investigators to lead to tumor rejection and subsequent anti-tumor immunity. Our results would suggest that tumor antigens might be considered to be weak agonists and that the normal immune system does not present tumor antigens with sufficient co-stimulation to activate T cells. Tumor antigens do not come with the associated ‘danger’ signals common to infectious agents that result in the induction of B7 expression. Presentation of weak tumor antigens by the tumor cell in the context of high co-stimulation may permit the initiation of an anti-tumor response.

In conclusion, studying the role of different parameters that influence T cell activation and differentiation is critical to our understanding of the functional outcome of a T cell response. Such studies may provide invaluable information for shifting a pathogenic response into one that is protective. In the case of an autoreactive T cell repertoire, both the size and the affinity of the repertoire are reduced as only low-affinity T cells that survive negative selection are able to reach the periphery. Since autoantigen is always present, our data would suggest that changes in the level of expression of co-stimulatory molecules such as B7, particularly in the target organ, may play a critical role in activation and expansion of an autoreactive T cell from a protective (Tc1/Tc2) to a pathogenic (Tc1) response and induction of autoimmune disease.

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Abbreviations

APC antigen-presenting cell
APL altered peptide ligand
CFA complete Freund’s adjuvant
CHO Chinese hamster ovary
MFI mean fluorescence intensity
PLP proteolipid protein
TNF tumor necrosis factor

References


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