CD148: A Receptor-Type Protein Tyrosine Phosphatase Involved in the Regulation of Human T Cell Activation

Stuart G. Tangye, Joseph H. Phillips, Lewis L. Lanier, Jan E. de Vries and Gregorio Aversa

*J Immunol* 1998; 161:3249-3255; 
http://www.jimmunol.org/content/161/7/3249
CD148: A Receptor-Type Protein Tyrosine Phosphatase Involved in the Regulation of Human T Cell Activation

Stuart G. Tangye, Joseph H. Phillips, Lewis L. Lanier, Jan E. de Vries, and Gregorio Aversa

Following ligation of the TCR and costimulatory molecules such as CD28, T cells proliferate and secrete cytokines. Several other cell surface molecules have been identified that are capable of augmenting activation mediated via the TCR. These include CD2, CD27, CD40 ligand, and signaling lymphocytic activation molecule. Here, we have characterized the expression and function of CD148, a recently identified receptor-type protein tyrosine phosphatase. CD148 is expressed at low levels on resting T cells, but is up-regulated following in vitro activation. Cross-linking CD148 with immobilized anti-CD148 mAb induced vigorous proliferation of anti-CD3 mAb-activated, highly purified peripheral blood T cells in an IL-2-dependent, cyclosporin A-sensitive manner. This effect was greatest after 8 days of in vitro culture, suggesting that this molecule is involved in the latter stages of a T cell response. CD148-induced proliferation was significantly greater for CD8+ T cells than for CD4+ T cells. Thus, CD148 is a receptor-type protein tyrosine phosphatase involved in the activation of T lymphocytes. The Journal of Immunology, 1998, 161: 3249–3255.

Optimal T cell activation requires signals delivered via not only the Ag receptor, but also by the engagement of costimulatory molecules. CD28 is the major costimulatory molecule expressed by T cells. Ligation of CD28 by its ligands CD80 and CD86, expressed on APCs, plays a critical role in the development of Ag-specific T cell responses. This is achieved not only by inducing vigorous T cell proliferation, but also by enhancing T cell survival by up-regulating the expression of bcl-xL and inducing the secretion of cytokines (reviewed in Refs. 1–3). Although T cells obtained from CD28-deficient mice exhibit impaired proliferative responses, these cells are still capable of proliferating in response to certain stimuli (4, 5). This suggests that additional T cell surface molecules exist that are capable of providing costimulatory signals to Ag-activated T cells. Indeed, several T cell surface molecules have been identified that are capable of augmenting anti-CD3-induced T cell proliferation. These include molecules that are either constitutively expressed on T cells, such as CD2 (6), CD9 (7), LFA-1 (8), CD27 (9), and CD47 (10), or molecules expressed following activation, such as CD40 ligand (11) and signaling lymphocytic activation molecule (SLAM) (12, 13).

We have generated a mAb (A3) that has been clustered as CD148 (13). CD148 (also known as human protein tyrosine phosphatase-η or density-enhanced protein tyrosine phosphatase-1) is a recently identified receptor-type protein tyrosine phosphatase (14, 15). The cloned product is a 180- to 250-kDa polypeptide with 8 to 10 fibronectin type III motifs in its extracellular domain, a single transmembrane domain, and one catalytic phosphatase domain within the intracellular domain (13, 14). CD148 mRNA and/or protein have been detected in fibroblasts (14) and some hemopoietic cell lines (14, 16), yet its expression in lymphoid cells remains uncharacterized. Using the anti-CD148 mAb, we have analyzed the distribution of CD148 on leukocyte populations and assessed the role of this protein tyrosine phosphatase in T cell activation. Our results demonstrate that CD148 is an inducible molecule expressed by activated T cells and that ligating CD148 on anti-CD3 mAb-activated T cells induces high levels of proliferation. Proliferation was even observed for highly purified T cells that did not respond to anti-CD3 mAb alone. These data suggest that the phosphatase activity of CD148 may be involved in the regulation of T cell activation.

Materials and Methods

Abs, cytokines, and reagents

The hybridoma cell line secreting the A3 mAb was generated by fusing NS-1 cells with spleenocytes from BALB/c mice that had been repeatedly immunized with PHA-stimulated human PBMC. The isotype of A3 was IgG1 κ. A3 was labeled with FITC using standard protocols. The following mAbs were used in this study: fluoroconjugated-mAb and unconjugated-mAb specific for CD3, CD4, CD8, CD14, CD16, CD19, CD20, CD28, CD45RA, CD45RO, CD56, and CD57 (Becton Dickinson, San Jose, CA); goat anti-mouse Ig (Jackson ImmunoResearch Laboratories, West Grove, PA); Spv-T3b (anti-CD3) (17); control IgG1 (MOPC-31; Pharmingen, La Jolla, CA); and neutralizing rat anti-human IL-2 (17H12, IgG2a) and a control rat IgG2a mAb (anti-β-galactosidase; provided by J. Abrams, DNAX, Palo Alto, CA). All mAbs used for functional studies were purified as previously described (12). IL-2 and IL-15 were purchased from R&D Systems (Minneapolis, MN); PHA was purchased from Sigma (St. Louis, MO).

Cells

T cells were isolated from the peripheral blood of healthy donors (Stanford Blood Bank, Stanford, CA) by negative selection using mAb specific for CD14, CD16, CD19, CD20, and CD56 and sheep anti-mouse Ig Dynalbeads (Dyna, Oslo, Norway) (18). The recovered cell population was typically 85% to 97% T cells. Highly purified peripheral blood T cells were obtained by negative cell sorting using a FACS Vantage or a FACStarPlus (Becton Dickinson) after staining the cells with PE-labeled anti-CD14, anti-CD16, anti-CD19, anti-CD20, and anti-CD56 mAbs. On reanalysis, the sorted T cells were >97.5% CD3+. Peripheral blood CD4+ and CD8+ T cells were also obtained by negative cell sorting after staining the cells...
with the same PE-labeled mAbs as those described above and including either PE-anti-CD4 or PE-anti-CD8 mAb. The sorted T cells were >96% CD4+ and >85% CD8+. Human T and NK cell clones were generated as previously described (19). The cell lines used in this study included EBV-transformed B cell lines JY, MoB, and 721.221; the Burkitts lymphoma cell line Ramos; the monocyteoid cell line U937; the immature NK leukemic cell line YT2C2; the erythroid leukemic cell line K562; the promyelocytic leukemic cell line HL60; and the human T cell lines Jurkat and HPB-ALL. All cell lines were cultured at 37°C in 5% CO2 in RPMI 1640 tissue culture medium (JRH Biosciences, Lenexa, KS) supplemented with 10% FBS, penicillin, streptomycin, and L-glutamine, hereafter referred to as complete medium.

**Immunofluorescent staining**

PBMC were incubated with PE-anti-CD3, PE-anti-CD4, PE-anti-CD8, PE-anti-CD14, PE-anti-CD20, or PE-anti-CD56 mAb and FITC-anti-CD148 or FITC-IgG1. The expression of CD148 on subsets of PBMC was determined by gating on the PE-positive cells and assessing the fluorescence of the population of cells incubated with FITC-anti-CD148 compared with the fluorescence of cells incubated with FITC-IgG1. The expression of CD148 on T cell subsets was determined by three-color immunofluorescent staining using PE-anti-CD28, PerCP-anti-CD4 or -anti-CD8, and FITC-IgG1 or anti-CD148 or using PerCP-anti-CD3, PE-anti-CD45RA or anti-CD45RO, and FITC-IgG1 or anti-CD148. Expression of CD148 was assessed by gating on the PerCP-positive cells. Surface staining was measured on a logarithmic scale. Five to ten thousand events were collected per sample, and the data were analyzed using the CellQuest software program (Becton Dickinson).

**Cell cultures**

Ninety-six-well round-bottom culture plates (Costar, Cambridge, MA) were coated with goat anti-mouse Ig (5 μg/ml), in 0.05 M carbonate buffer, pH 9.6) at 37°C for 4 h. Anti-CD148 mAb or a control IgG1 mAb (10 μg/ml) were added to the wells and incubated overnight at 4°C. Anti-CD3 and anti-CD28 mAbs were added at a final concentration of 1 μg/ml. In some experiments, soluble control IgG1 mAb, soluble anti-CD148 mAb (10 μg/ml), neutralizing anti-human IL-2 mAb, control rat IgG2a mAb (20 μg/ml), or Csa (Calbiochem, San Diego, CA) were added to the wells. For proliferation studies, 2.5 to 5 × 10^4 T cells were added to each well of a 96-well plate in a total volume of 200 μl of complete medium and then cultured for 2 to 8 days at 37°C in 5% CO2. Proliferation was determined by assessing the incorporation of [3H]thymidine (1 μCi/well; Amersham, Arlington Heights, IL) by triplicate or quadruplicate cultures of T cells during the final 18 h of the culture period. Incorporation of [3H]thymidine was measured as counts per minute by liquid scintillation counting with a beta counter (Pharmacia-LKB-Wallac, Turku, Finland). For cytokine secretion, 10^5 sort-purified peripheral blood T cells were cultured in 200 μl of Yssel’s medium (20) supplemented with 10% FBS, penicillin, and streptomycin with anti-CD3 mAb (1 μg/ml) in the presence or absence of immobilized control IgG1, immobilized anti-CD148 mAb (10 μg/ml), or soluble anti-CD28 mAb (1 μg/ml). Supernatants were harvested from five replicate wells after 48 h of culture, and the levels of secreted IL-2, IL-10, granulocyte-macrophage CSF, TNF-α, and IFN-γ were determined by ELISAs, as previously described (18, 21).

**Results**

**Expression of CD148 on human cells**

To assess the expression of CD148, mononuclear cells from human peripheral blood were incubated with FITC-anti-CD148 mAb in combination with PE-labeled mAb specific for T cells, B cells, NK cells, and monocytes and were analyzed by flow cytometry. CD148 was expressed at different densities on all leukocyte populations examined (Fig. 1). Monocytes expressed the highest level of CD148, whereas expression was lowest on CD4+ T cells. Expression on B cells, CD8+ T cells, and NK cells was intermediate to that on CD4+ T cells and monocytes. The differential expression of CD148 on CD4+ and CD8+ T cells was consistently observed for all donor PBMC samples and explains the broad staining pattern observed for total T cells (Fig. 1). Expression of CD148 did not differ on subsets of peripheral blood T cells defined by the expression of CD45RA, CD45RO, or CD28 (data not shown). CD148 expression on CD4+ T cells, CD8+ T cells, NK cells, and monocytes was similar regardless of whether the mononuclear cells were obtained from peripheral blood, spleen, or cord blood (data not shown). CD148 was expressed at elevated levels on CD4+ T cell clones, CD8+ T cell clones, and NK cell clones (Table I). CD148 was also expressed on the U937, YT2C2, K562, and HL60 cell lines. In contrast, this molecule was either absent or was expressed at only very low levels on transformed B and T cell lines (Table I).

**CD148 expression is up-regulated following activation**

PBMC were cultured with PHA, anti-CD3 mAb, or the cytokines IL-2 or IL-15, and the expression of CD148 was assessed on CD4+ and CD8+ T cells at different times following activation in vitro. Expression of CD148 on either CD4+ or CD8+ T cells remained unchanged when the cells were cultured without any exogenous stimuli (Fig. 2). Expression of CD148 was up-regulated on all T cells following activation with PHA or anti-CD3 mAb (Fig. 2a). Kinetic studies indicated that CD148 expression was increased 6 to 12 h after activation and that CD148 expression continued to increase until maximum expression levels were observed after 72 to 96 h (data not shown). Expression of CD148 on CD4+ and CD8+ T cells was also increased following culture with IL-2 or IL-15 (Fig. 2b). Exposure to IL-2 for 4 days up-regulated
CD148 expression on 20.8 ± 7.1% of CD4+ T cells and 45 ± 8.5% of CD8+ T cells (mean ± SD of four independent experiments). Culture with IL-15 had a similar effect as IL-2, increasing CD148 expression on 17.6 ± 6.4% of CD4+ T cells and 47.1 ± 14.3% of CD8+ T cells (Fig. 2b).

**Cross-linking CD148 augments proliferation of activated T cells**

To determine the role of CD148 in T cell activation, peripheral blood T cells were cultured with anti-CD3 mAb in the absence or

*FIGURE 2. CD148 expression is increased following T cell activation. PBMC were cultured in the absence or the presence of (a) PHA (1 µg/ml) or anti-CD3 mAb (1 µg/ml) for 2 days, or (b) IL-2 (100 U/ml) or IL-15 (5 ng/ml) for 4 days. The expression of CD148 on CD4+ (upper panel) and CD8+ T cells (lower panel) was determined as described in Figure 1. The bold histogram shows the expression of CD148 in the presence of the indicated stimulus; the thin histogram represents the fluorescence of cultured cells incubated with an isotype control mAb.*

*FIGURE 3. Ligation of CD148 enhances the proliferation of anti-CD3-activated human peripheral blood T cells. Peripheral blood T cells (90% CD3+ were cultured with anti-CD3 mAb alone (1 µg/ml; ○) or in the presence of soluble control IgG1 mAb (10 µg/ml; ●), soluble anti-CD148 mAb (10 µg/ml; ○), immobilized control IgG1 mAb (10 µg/ml; ▲), immobilized anti-CD148 mAb (10 µg/ml; ■), or anti-CD28 mAb (1 µg/ml; Δ). Proliferation was assessed at the times indicated by determining the incorporation of [3H]thymidine during the final 18 h of culture. Each point represents the mean ± SD of quadruplicate samples. This result is representative of five independent experiments.*
Table II. Ligation of CD148 increases the recovery of anti-CD3 mAb-activated viable T cells

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Anti-CD3</th>
<th>+ cIgG</th>
<th>+ Anti-CD148</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.6 ± 0.5</td>
<td>1.54 ± 0.41</td>
<td>4.26 ± 0.7</td>
</tr>
<tr>
<td>2</td>
<td>0.84 ± 0.14</td>
<td>0.57 ± 0.09</td>
<td>3.3 ± 0.2</td>
</tr>
<tr>
<td>3</td>
<td>0.62 ± 0.17</td>
<td>0.52 ± 0.1</td>
<td>2.55 ± 0.16</td>
</tr>
</tbody>
</table>

* Peripheral blood T cells (85–97% CD3+) purified from three different donors were cultured with anti-CD3 mAb alone (1 μg/ml) or in the presence of immobilized control IgG1 (10 μg/ml) or immobilized anti-CD148 mAb (10 μg/ml).

* The number of viable cells was determined after 10 days by trypan blue exclusion.

* Each value represents the mean ± SD of quadruplicate samples. The initial density of the cultured cells was 0.25 × 10⁶/ml.

recovered from cultures stimulated with anti-CD3 mAb and immobilized anti-CD148 mAb than from cultures stimulated with anti-CD3 and immobilized control IgG1 mAb or with anti-CD3 alone (Table II).

Ligation of CD148 induces proliferation of highly purified, anti-CD3 mAb-activated T cells

Because the cell preparations used in the above studies contained only about 90% CD3+ cells, the ability of anti-CD148 mAb to enhance the proliferation of anti-CD3 mAb-activated T cells could be an indirect effect, resulting from the activation of other CD148+ cells, such as monocytes. To assess this possibility, highly purified T cells (> 97.5% CD3+) obtained by negative cell sorting were cultured with anti-CD3 mAb in the absence or the presence of an immobilized control IgG1 mAb or anti-CD148 mAb. Anti-CD3 mAb alone or anti-CD3 plus control IgG1 mAb failed to induce the proliferation of purified T cells (Fig. 4). However, ligation of CD148 by immobilized anti-CD148 mAb induced high levels of proliferation of anti-CD3 mAb-activated T cells (Fig. 4). It is well known that activation via CD3 and CD28 is capable of inducing maximal T cell proliferation (1–3). Comparison of the ability of cross-linking CD28 or CD148 to induce T cell proliferation indicated that proliferation of highly purified anti-CD3 mAb-activated

FIGURE 4. Ligation of CD148 induces the proliferation of anti-CD3-activated, highly purified T cells. Sort-purified peripheral blood T cells from five different donors (>97.5% CD3+) were cultured with anti-CD3 mAb alone (1 μg/ml; □) or in the presence of immobilized control IgG1 mAb (10 μg/ml; □), immobilized anti-CD148 mAb (10 μg/ml; □), or soluble anti-CD28 (1 μg/ml; □). Proliferation was assessed after 5 or 6 days by determining the incorporation of [3H]thymidine during the final 18 h of culture. The proliferation of cells stimulated with anti-CD3 mAb alone or with anti-CD3 plus immobilized control IgG was <1500 cpm. Each point represents the mean ± SD of triplicate or quadruplicate samples.

FIGURE 5. Cross-linking CD148 preferentially activates CD8+ T cells. Total T cells or sort-purified CD4+ and CD8+ peripheral blood T cells were cultured with anti-CD3 mAb alone (1 μg/ml; □) or in the presence of immobilized control IgG1 mAb (10 μg/ml; □), immobilized anti-CD148 mAb (10 μg/ml; □), or soluble anti-CD28 mAb (1 μg/ml; □). Proliferation was assessed after 5 or 6 days by determining the incorporation of [3H]thymidine during the final 18 h of culture. Each point represents the mean ± SD of triplicate or quadruplicate samples. Data presented are representative of three independent experiments.

T cells mediated by immobilized anti-CD148 mAb was about 25 to 75% of that mediated by anti-CD28 for the five different donors examined. These results indicate that the combination of anti-CD148 mAb and anti-CD3 mAb is a potent activator of proliferation of highly purified T cells.

Differential responses of T cell subsets to anti-CD148 mAb

Expression of CD148 was higher on resting CD8+ T cells than on CD4+ T cells. Additionally, a higher percentage of CD8+ T cells up-regulated CD148 expression in response to IL-2 and IL-15 than did CD4+ T cells. To assess whether these differences played a role in the in vitro response of T cells, CD4+ and CD8+ T cell subsets were purified from peripheral blood and were cultured with anti-CD3 mAb in the absence or the presence of immobilized control IgG1 mAb, immobilized anti-CD148 mAb, or anti-CD28 mAb. Purified CD4+ and CD8+ T cells exhibited minimal or only a low level of proliferation in response to anti-CD3 mAb alone or in the presence of immobilized control IgG1 mAb (Fig. 5). However, anti-CD3 mAb-activated CD4+ and CD8+ T cells proliferated when CD148 was ligated by the immobilized anti-CD148 mAb (Fig. 5). Although both T cell populations proliferated, the response of CD8+ T cells was consistently several-fold greater than that of CD4+ T cells. As a comparison, the proliferation of CD4+ T cells in response to anti-CD3 plus anti-CD148 mAb was only about 10% of that induced by anti-CD3 plus anti-CD28 mAb, while the proliferation of CD8+ T cells activated with anti-CD3 plus anti-CD148 mAb was 50 to 100% of the proliferative response induced by anti-CD3 plus anti-CD28 mAb. Thus, the peripheral blood cell type that predominates in the response to anti-CD3 plus anti-CD148 mAb appears to be CD8+ T lymphocytes.

The anti-CD148 mAb-mediated increase in proliferation is IL-2 dependent and CsA sensitive

To determine whether ligation of CD148 increased T cell proliferation by an IL-2-dependent or a CsA-sensitive mechanism, peripheral blood T cells were activated in the presence of neutralizing anti-IL-2 mAb or CsA. In the presence of immobilized anti-CD148 mAb, T cell proliferation induced by anti-CD3 was increased up to 30-fold (data not shown). However, when anti-IL-2 mAb was included in the culture, the enhanced levels of proliferation of anti-CD3 mAb-activated
T cells induced by the anti-CD148 mAb were reduced by 73 ± 11.5% (Fig. 6; mean ± SEM; n = 4). In contrast, culture in the presence of an isotype control rat IgG2a mAb (clgG2a; 20 μg/ml), rat anti-human IL-2 mAb (anti-IL-2; 20 μg/ml), or CsA (10 or 100 ng/ml). Proliferation was assessed after 5 days by determining the incorporation of [3H]thymidine during the final 18 h of culture. Each point represents the mean ± SD of quadruplicate samples. This result is representative of four independent experiments.

**Effect of anti-CD148 mAb on cytokine secretion by activated T cells**

The effect that cross-linking CD148 had on cytokine secretion was assessed using sort-purified T cells from three separate donors. Activation with anti-CD3 mAb alone induced a low level of secretion of IL-2, IL-10, IFN-γ, TNF-α, and granulocyte-macrophage CSF (Table III). Interestingly, this response was unaffected by cross-linking CD148 on the activated T cells. In contrast, costimulation with anti-CD28 mAb increased the level of secreted cytokines by 3- to 20-fold (Table III). The inability of the anti-CD148 mAb to influence cytokine secretion was not due to inefficient activation because the cells responded with high levels of proliferation when assessed after 5 days of in vitro culture (data not shown).

**Discussion**

CD148 is a receptor-type protein tyrosine phosphatase expressed by all resting leukocytes. The levels of expression of CD148 were greatest on monocytes, intermediate on NK cells, B cells, and CD8+ T cells, and lowest on CD4+ T cells. Expression of CD148 could be increased on peripheral blood T cells following in vitro activation with PHA or anti-CD3 mAb. Consistent with this, elevated amounts of phosphatase activity could be precipitated from lysates of activated T cells, compared with unstimulated T cells, by the anti-CD148 mAb (data not shown). The increase in expression of CD148 by in vitro activation with PHA and anti-CD3 mAb followed kinetics similar to those previously described for the mitogen-inducible phosphatases PAC-1 and hemopoietic protein tyrosine phosphatase (23, 24), suggesting that these different phosphatases may act at similar times during T cell activation. CD148 could also be up-regulated on 20 to 50% of peripheral blood T cells in the presence of IL-2 or IL-15. The ability of IL-15 to up-regulate CD148 on peripheral blood T cells to a comparable extent as IL-2 is consistent with the overlapping biologic functions reported for these cytokines (25). Analysis of subsets of CD4+ and CD8+ T cells did not reveal preferential up-regulation of CD148 on subsets defined by the expression of CD28 or CD57. Consequently, the significance of the differential up-regulation of CD148 on CD8+ and CD4+ T cells is presently unknown.

The finding that expression of CD148 is increased on T cells following activation suggested that this molecule may be involved in the functional regulation of activated T cells. Indeed, ligation of CD148 on T cells by immobilized, but not soluble, anti-CD148 mAb greatly augmented the in vitro proliferative response induced by ligation of the TCR by anti-CD3 mAb. More importantly, anti-CD148 mAb induced proliferation of highly purified, FACs-sorted T cells that had been activated with anti-CD3 mAb, which was unable to drive T cell proliferation in the absence of costimulatory signals. The combination of anti-CD3 plus anti-CD148 mAb often induced a proliferative response in highly purified T cells that approached that induced by anti-CD3 plus anti-CD28 mAb. This indicates that the combination of anti-CD3 plus anti-CD148 mAb is a potent T cell activator and suggests that CD148 engagement can initiate T cell proliferation in the absence of CD28 costimulation. CD148-mediated T cell proliferation was also found to be dependent on the production of endogenous IL-2 and was sensitive

### Table III. Cytokine secretion by anti-CD3-activated T cells is not affected by ligation of CD148

<table>
<thead>
<tr>
<th>Cytokine</th>
<th>Anti-CD3</th>
<th>+ IgG</th>
<th>+ Anti-CD148</th>
<th>+ Anti-CD28</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>5,278 ± 601</td>
<td>4,200 ± 1,110</td>
<td>5,210 ± 610</td>
<td>16,456 ± 2,120</td>
</tr>
<tr>
<td>IL-10</td>
<td>1,036 ± 420</td>
<td>600 ± 380</td>
<td>340 ± 200</td>
<td>2,785 ± 860</td>
</tr>
<tr>
<td>IFN-γ</td>
<td>182 ± 80</td>
<td>81 ± 23</td>
<td>291 ± 91</td>
<td>5,925 ± 1,790</td>
</tr>
<tr>
<td>TNF-α</td>
<td>3,686 ± 180</td>
<td>4,076 ± 222</td>
<td>4,207 ± 175</td>
<td>7,861 ± 600</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>432 ± 70</td>
<td>700 ± 310</td>
<td>1,437 ± 377</td>
<td>7,211 ± 600</td>
</tr>
</tbody>
</table>

* Sort-purified peripheral blood T cells (>97.5% CD3+) from three different donors were cultured with anti-CD3 mAb alone (1 μg/ml) or in the presence of immobilized control IgG1 (10 μg/ml), immobilized anti-CD148 mAb (10 μg/ml), or anti-CD28 mAb (1 μg/ml).

* The secretion of IL-2, IL-10, IFN-γ, TNF-α, and GM-CSF was determined by cytokine-specific ELISAs after 48 h of culture.

* Each value represents the mean ± SEM of five replicate supernatants obtained from three separate donors.
to treatment with CsA. Analysis of T cell subsets indicated that the response of CD8+ T cells to activation with anti-CD3 and anti-CD148 mAb was much greater than that of CD4+ T cells. These differences in proliferation of CD4+ and CD8+ T cells were even more impressive when it was considered that the CD8+ CD28+ T cell subset, which can comprise up to 40% of total CD8+ T cells (26), failed to proliferate in response to anti-CD3 plus anti-CD148 mAb (data not shown). CD4+ T cells and CD8+ T cells have previously been reported to preferentially respond to activation via CD40 ligand (11) and Fas ligand (27), respectively. Similarly, CD27 is a costimulatory molecule for CD45RA+ T cells, but not CD45RO+ T cells (9, 28). These differences in responses of T cell subsets correlated with the differential surface expression of such molecules. This is consistent with our observation that expression of CD148 is greater on unstimulated CD8+ T cells than on CD4+ T cells, and that more CD8+ T cells up-regulate CD148 expression in response to IL-2 and IL-15.

Cell surface molecules previously found to enhance the proliferation of Ag receptor-activated T cells are often also capable of enhancing cytokine secretion by the activated T cells (9, 11, 12). It was therefore surprising that there was no detectable increase in the level of cytokine secreted by T cells activated with anti-CD3 plus anti-CD148 mAb. This, however, is inconsistent with the finding that the increased proliferation mediated by anti-CD148 mAb was dependent on the production of endogenous IL-2. Autocrine consumption of cytokines by the proliferating T cells may at least in part account for the inability to detect an increase following ligation of CD148. The ability of anti-IL-2 mAb to reduce CD148-induced proliferation suggests that at least IL-2 production is enhanced by anti-CD3 mAb-activated T cells in the presence of immobilized anti-CD148 mAb.

Although T cell proliferation induced by anti-CD3 mAb in combination with anti-CD148 mAb was comparable to that induced by anti-CD3 plus anti-CD28 mAb, it appears that the responses of anti-CD3 mAb-activated T cells to these two different mAbs are distinct. Differences were evident for 1) the kinetics of the T cell response; 2) the sensitivity of proliferation induced by anti-CD148 mAb, but not anti-CD28 mAb (22), to CsA; 3) the inability of anti-CD148 mAb to have any apparent effect on cytokine secretion after 2 days of in vitro culture; and 4) the ability of CD8+ T cells to exhibit greater levels of proliferation in response to anti-CD148 mAb.

To date, the best characterized protein tyrosine phosphatases identified to play important roles in Ag receptor-mediated signal transduction are CD45 and SH2-containing protein tyrosine phosphatase-1, protein tyrosine phosphatases that are constitutively expressed by all hematopoietic cells. Analysis of cell lines and of mice deficient in these phosphatases have indicated that CD45 is necessary and sufficient for initiation of Ag receptor-mediated signal transduction in both B and T cells (29–34). In contrast, SH2-containing protein tyrosine phosphatase-1 can negatively regulate Ag receptor-mediated signal transduction (35–39). Thus, phosphatases can act to either initiate or terminate cellular responses. Presently, the mechanism by which anti-CD148 mAb amplifies the proliferative response of anti-CD3 mAb-activated T cells is unknown. Ligating the molecule with anti-CD148 mAb, which may mimic the interaction with its physiologic ligand, may deliver a positive signal. Alternatively, ligating CD148 may inhibit a negative signal by inducing dimerization of the phosphatase domain of CD148 (40, 41). This would be consistent with the ability to inhibit signal transduction mediated via CD45 by ligand-induced dimerization of its extracellular domain (42). The outcome of either delivering a positive or inhibiting a negative signal would be reflected by an elevated T cell response. Elucidation of the mechanism is presently under investigation. In conclusion, CD148 is a receptor-type protein tyrosine phosphatase expressed on all leukocytes that is up-regulated following in vitro stimulation. Thus, CD148 is a T cell activation molecule. The phosphatase activity of CD148 may be involved in regulating the expansion of in vivo-activated T cells, particularly CD8+ T cells. The finding that this molecule is expressed at elevated levels on monocytes as well as on resting and activated NK cells suggests that CD148 may have a role in the biologic responses of different populations of mononuclear cells and that its primary function is not restricted to T cells.

Acknowledgments

We thank Dr. Jim Cupp, Eleni Callas, Erin Murphy, and Dixie Pollakoff for cell sorting, and Sasha Lazetic and Patricia Larens for providing cell lines and clones.

References


