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Expression Characteristics and Stimulatory Functions of CD43 in Human CD4\(^+\) Memory T Cells: Analysis Using a Monoclonal Antibody to CD43 That Has a Novel Lineage Specificity\(^1\)

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We have used HSCA-2, an mAb that recognizes a sialic acid-dependent epitope on the low molecular mass (~115-kDa) glycoform of CD43 that is expressed in resting T and NK cells, to examine the expression characteristics and stimulatory functions of CD43 in human CD4\(^+\) memory T cells. Having previously reported that the memory cells that respond to recall Ags in a CD4\(^+\)/CD45RO\(^+\) T cell population almost all belong to a subset whose surface CD43 expression levels are elevated, we now find that exposing these same memory T cells to HSCA-2 mAb markedly increases their proliferative responsiveness to recall Ags. We think it unlikely that this increase in responsiveness is a result of CD43-mediated monocyte activation, especially given that the HSCA-2 mAb differs from all previously used CD43 mAbs in having no obvious binding specificity for monocyte CD43. Predictably, treatment with HSCA-2 mAb did not lead to significant recall responses in CD4\(^+\)/CD45RO\(^+\) T cells, whose CD43 expression levels were similar to or lower than those of naïve cells. Other experiments indicated that the HSCA-2 mAb was capable of enhancing the proliferative responsiveness of CD4\(^+\) memory T cells that had been exposed to polyclonal stimulation by monocyte-bound CD3 mAb and could also act in synergy with CD28 mAb to enhance the responsiveness of CD4\(^+\) T cells to CD3 stimulation. Taken together, these findings suggest that the CD43 molecules expressed on CD4\(^+\) memory T cells may be capable of enhancing the costimulatory signaling and hence providing accessory functions to TCR-mediated activation processes. The Journal of Immunology, 2004, 172: 7246–7253.

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We have recently described how HSCA-2, a novel CD43 mAb, can be used for the classification of human CD4+CD45RO+ memory T cells into three subsets on the basis of differences in their CD43 expression (31). In this classification, cells of the first of the three subsets (the M1 subset) express elevated levels of CD43, whereas cells of the M2 subset express CD43 levels similar to those of naive cells, and cells of the M3 subset express reduced CD43 levels. We also found that the M1 subset contains the highest proportion of recall Ag-reactive precursors and secretes substantially more IFN-γ and IL-4. The majority of effector memory T cells (CCR7-), among them some memory T cells (CD27-CD45RO+CD44hi), may also contain central memory T cells. The M2 subset cells are less mature memory cells that retain longer telomeres than do cells of the M1 and M3 subsets, and their memory functionality (including recall Ag reactivity) appears to be marginal (31). The M3 subset consists of cells that are anergic to TCR-mediated stimulation (including recall Ag reactivity) and prone to apoptosis (31). As the level of CD43 expression decreases on the M2 subset cells, the memory functionality of these molecules in the proliferative responses of CD4+ T cells is reduced. This is illustrated by the fact that the subset may also contain central memory T cells. The M2 subset cells express CD43 levels similar to those of the M3 subset and, as such, may perhaps be considered a subset of the M3 subset. Moreover, the M2 subset cells are even less reactive than the M3 subset cells, as demonstrated in the proliferative assays described below.

Materials and Methods

Production of HSCA-2 mAb

The HSCA-2 hybridoma is a product of the fusion of NS1 mouse myeloma cells with splenocytes from BALB/c mice immunized by injection of human KG-1 cells (31). Immunization, fusion, selection, and cloning protocols were essentially as described previously (33). Hybridoma supernatants were initially screened for reactivity with KG-1 cells by indirect immunofluorescence. The HSCA-2 hybridoma was selected for further study because of its unique specificity of reactivity with PBMC and cord blood CD4+ stem cells. Isotype characterization showed that the HSCA-2 mAb was of the IgG1 subclass. Ascites fluid was obtained from SCID mice injected with the HSCA-2 hybridoma. After purification from ascites fluid by DE52 ion exchange chromatography, HSCA-2 mAb was labeled with FITC (Sigma-Aldrich, St. Louis, MO) for flow cytometry. Fab of HSCA-2 mAb were prepared by digestion with papain (34). This mAb was labeled for participation in the Eighth International Workshop and Conference on Human Leukocyte Differentiation Ags (to be held in Adelaide, Australia).

Other mAbs

Unconjugated CD28 mAb (clone CD28.2) (35), used for T cell culture, was purchased from Coulter-Immunotech (Marseille, France). Unconjugated and FITC-conjugated CD43 mAbs, DFT-1, and L10 (36), and CD14 (37), were obtained from Coulter-Immunotech, Caltag Laboratories (Burlingame, CA), and BD Pharmingen (San Diego, CA), respectively. PE-labeled CD4, CD8, CD14, CD19, and CD56 mAbs and PerCP-labeled CD4 and CD8 mAbs were purchased from BD Biosciences (San Jose, CA). PE-labeled CD45RO mAb was obtained from Caltag Laboratories.

Transfection of CD43 cDNA

Total RNA of KG-1 cells was isolated with TRIzol reagent (Invitrogen, Carlsbad, CA). First-strand cDNA, primed with oligo(dT)12, was synthesized using SuperScript II reverse transcriptase (Invitrogen). CD43 cDNA was PCR-amplified with the primers 5'-ctttgctctggcctgctggc-3' and 5'-catgggtagtggcctgcta-3' using Advantage cDNA polymerase mix (Clontech Laboratories, Palo Alto, CA) and cloned into pcR2.1 TA cloning vector (Invitrogen). The sequence-verified clone was recloned into the EcoRI site of pIREsneo (Clontech Laboratories) and designated pIREsneo HCD43. Subsequently, 30 μg of pIREsneo HCD43 or pIREsneo negative control (neomycin) was electroporated into 2 × 10⁶ HeLa cells in 200 μl of PBS containing 10% FCS (Pansorbin, Bio-Rad, Hercules, CA). Cells were treated with 1 μg/ml G418 for 2 wk. Drug-resistant colonies were selected and expanded to confirm CD43 expression by flow cytometry. HeLa transfected cells expressing high levels of CD43 were isolated by a cell sorter for additional experiments.

Cell preparations and flow cytometry

For direct immunofluorescence of cultured cell lines, 2 × 10⁶ CD43- or mock-transfected HeLa and KG-1 cells were stained with 1 μg of FITC-labeled HSCA-2, DFT-1, and MOPC21 mAbs for 45 min on ice. For the analyses of CD43 glycoepitopes, 2 × 10⁶ KG-1 cells were treated with neuraminidase (0.1 U/ml in PBS) for 30 min at 37°C. For competitive inhibition, KG-1 cells were pretreated with various amounts of HSCA-2 or DFT-1 mAbs (12.5–200 μg/ml) for 1 h on ice and then stained with FITC-labeled HSCA-2 and DFT-1 mAbs for 45 min. FACScan (BD Biosciences) was used for flow cytometric analyses.

For flow cytometry of human blood cells, PBMCs from healthy adult volunteer donors (n = 6) and cord blood mononuclear cells (n = 3) were isolated by density centrifugation in Ficoll-Hypaque (density, 1.077 g/ml; ICN Biomedical, Aurora, OH). Granulocytes were isolated by double-density centrifugation in Ficoll-Hypaque (density, 1.077 and 1.119 g/ml; Wako Pure Chemical, Osaka, Japan) according to the manufacturer’s instructions. For isolation of monocytes, CD14+ cells were purified from PBMCs by positive enrichment using autoMACS (Miltenyi Biotec, Bergish Gladbach, Germany) according to the manufacturer’s instructions. Enriched monocytes also were used for immunoprecipitation and proliferation assay, as described below.

For single-color analysis of purified monocytes and granulocytes, cells were stained with FITC-labeled CD43 mAbs and analyzed by flow cytometry with a gate in a region for monocytes or granulocyte fractions on the forward and side light scatter profiles. For two-color analysis of lymphocytes, PBMCs were stained with PE-labeled CD4, CD8, CD19, and CD56 in combination with FITC-labeled CD43 mAbs. Cord blood mononuclear cells were stained with FITC-labeled CD43 and PE-labeled CD34 mAbs. For triple-color analysis, PBMCs were stained with FITC-labeled CD43, PE-conjugated CD45RO, and PerCP-labeled CD4 mAbs. CD4+ lymphocytes were gated on forward/side scatter and PerCP fluorescence. The proportions of CD4+ CD45RO- cells (RO− subset) and CD4+ CD45RO+ cells expressing high (M1 subset), intermediate (M2), and low (M3) levels of CD43 were measured by flow cytometry with FACScan (see Fig. 4).

For purification of activated CD4+ T cells, MACS-purified CD4+ T cells were stimulated with immobilized anti-CD3 mAb (OKT-3) in the presence of IL-2 (10 ng/ml) for 4 days in RPMI 1640 supplemented with 10% FCS. Immobilized CD3 mAb was prepared by binding OKT3 mAb (10 μg/ml in sodium bicarbonate buffer, pH 9.6) in 24-well plates at room temperature for 2 h, then washing the plates with RPMI 1640 supplemented with 10% FCS. For isolation of the four CD4+ T cell subsets, M1, M2, M3, and CD45RO−, CD4+ cells were purified by negative enrichment using MACS as described previously (31). MACS-purified CD4+ T cells were stained with FITC-labeled HSCA-2 and PE-labeled CD45RO mAbs. After incubation with propidium iodine at 10 μg/ml for 15 min to gate out dead cells, CD4+ T cells in the four subsets were sorted by a single laser cell sorter (FACSstar, BD Biosciences). During cell sorting, stained and sorted cell suspensions were maintained at 4°C by a cooling circulation system.

Cell proliferation assay

For proliferative response to recall Ags, PBMCs (5 × 10⁶ cells/well) in 96-well, flat-bottom plastic plates were stimulated with tuberculin purified protein derivative (PPD; 3 Connaught Laboratories, Ontario, Canada) or tetanus toxoid (TT; Calbiochem, La Jolla, CA) at 5 μg/ml. For total and subset CD4+ T cells, T cells (5 × 10⁶ cells/well) were stimulated with
these recall Ags in the presence of autologous monocytes (2.5 × 10^6 cells/well) that were previously isolated using autoMACS with anti-CD14 Ab (Miltenyi Biotec) and irradiated with x-ray at 30 Gy. The culture medium used for this assay was RPMI 1640 supplemented with 10% human serum. For proliferative responses to anti-CD3 mAb, total CD4+ T cells or sorted subset T cells were stimulated with various concentrations of soluble CD3 (OKT-3) mAb (0.0001–1 μg/ml) in the presence of autologous monocytes. The culture medium used for this assay was RPMI 1640 medium supplemented with 10% FCS.

The effects of CD43 (0.05–5 μg/ml) and CD28 mAbs (1 μg/ml) on cell proliferation were evaluated. Proliferation was measured on day 3 for CD3 T cells and on day 5 for PPD by adding [3H]thymidine (NEN, Boston, MA) to KG-1 cells, and on day 3 for CD3 (OKT-3) mAb (0.0001–1 μg/ml) in the presence of autologous monocytes. Proliferation was measured on day 3 for CD3 (OKT-3) mAb (0.0001–1 μg/ml) in the presence of autologous monocytes. The culture medium used for this assay was RPMI 1640 medium supplemented with 10% FCS.

Results

Characterization of CD43 epitopes recognized by HSCA-2 mAb

HSCA-2 mAb resembles the reference CD43 mAb DFT-1 in binding to HeLa cells stably transfected with CD43-cDNA, but not to their mock-transfected (CD43−) counterparts (Fig. 1A). HSCA-2 mAb also resembles the DFT-1 mAb in recognizing a neuraminidase-sensitive epitope on KG-1 cells (Fig. 1B). However, although the binding of HSCA-2 mAb to KG-1 cells was all but completely blocked by DFT-1 mAb, the binding of DFT-1 mAb was only ~90% blocked by HSCA-2 mAb even at the highest HSCA-2 mAb concentration (Fig. 1C). These findings may imply that the binding affinity of HSCA-2 mAb is lower than that of DFT-1 mAb. The HSCA-2 mAb immunoprecipitated a surface protein of ~115 kDa in KG-1 cells, whereas DFT-1 mAb immunoprecipitated both the 115-kDa protein and a minor protein with a higher molecular mass of ~125 kDa (Fig. 2A). Immunoprecipitation and blocking experiments with another characterized CD43 mAb, 1G10, confirmed the results with the DFT-1 mAb (data not shown). These results suggest that HSCA-2 mAb reacts with a

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**FIGURE 1.** Flow cytometric analyses of CD43 expression in CD43+ HeLa transfectant and KG-1 cells with HSCA-2 and DFT-1 mAbs. A, Direct immunofluorescence staining of mock-transfected (upper panel) and CD43-transfected HeLa cells (lower panel) with FITC-labeled HSCA-2 (thick line), DFT-1 (thin line), and control IgG1 (broken line) mAbs. B, Effect of neuraminidase treatment on the expression of CD43 in KG-1 cells. Nontreated (thick line) and treated (thin line) KG-1 cells were stained with FITC-labeled HSCA-2 (upper panel), DFT-1 (lower panel) mAbs. Treated cells also were stained with control IgG1 (broken line). C, Blocking of the binding of FITC-labeled HSCA-2 (left) and DFT-1 (right) mAbs to KG-1 cells by excess amounts of HSCA-2, DFT-1, or MOPC21 (IgG1 control) mAbs.

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**FIGURE 2.** Immunoprecipitation of 125I-labeled surface proteins from various types of white blood cells with HSCA-2, DFT-1, and MOPC21 (IgG1 control) mAbs. A, Immunoprecipitation of KG-1 cells and total PBMCs. B, Immunoprecipitation of CD14+, CD14−, CD4+, and activated CD4+ cells; 105-, 115-, 125-, and 135-kDa protein bands are indicated by an asterisk, arrows, open triangles, and closed triangles, respectively.
sialic acid-dependent epitope on the 115-kDa CD43 glycoform in KG-1 cells, but not with its equivalent on the 125-kDa glycoform.

Expression characteristics of CD43 in normal white blood cells

The particular CD43 epitopes recognized by the HSCA-2 or DFT-1 mAb (hereafter abbreviated to CD43(HSCA-2) or CD43(DFT-1)) in normal lymphoid and myeloid cell populations were analyzed by two-color flow cytometry (Fig. 3). The CD43(HSCA-2) and CD43(DFT-1) epitopes were expressed at similar levels in CD4<sup>+</sup> and CD8<sup>+</sup> T cell populations. Neither HSCA-2 mAb nor DFT-1 mAb reacted with resting CD19<sup>+</sup> B cells, whereas they both bound reasonably strongly to either PWM-activated or EBV-transformed B cells (data not shown). The majority of CD56<sup>+</sup> NK cells expressed both CD43(HSCA-2) and CD43(DFT-1) epitopes at high levels, whereas there was relatively little of the CD43(HSCA-2) epitope in the minor subpopulation of NK cells. DFT-1 mAb was found to bind quite strongly to both purified monocytes and granulocytes, whereas binding by the HSCA-2 mAb was weak enough to be described as nonspecific, as judged by the results of immunoprecipitation analyses (see Fig. 2B). We did not detect any increase in the level of either CD43(HSCA-2) or CD43(DFT-1) in cultured monocytes, even after the addition of LPS (data not shown). The HSCA-2 and DFT-1 mAbs both reacted quite strongly with cord blood CD43<sup>+</sup> cells (data not shown). Two previously used CD43 mAbs, 1G10 and L10, appeared to share the cell type specificities of the DFT-1 mAb (data not shown).

The results of experiments involving immunoprecipitation of 125I-labeled surface proteins from PBMCs, CD14<sup>+</sup> lymphoid cells, and CD4<sup>+</sup> T cells with CD43 mAbs revealed that the HSCA-2 mAb recognizes only 115-kDa proteins, whereas the DFT-1 mAb also reacts with 115-kDa proteins it can interact with a second minor, but higher molecular mass (~125 kDa), protein as well (Fig. 2); these findings mirror our previous findings with KG-1 cells (see above). Other findings include the fact that HSCA-2 mAb failed to immunoprecipitate any specific proteins from CD14<sup>+</sup> monocytes, unlike the DFT-1 mAb, which reacted with a 135-kDa protein (Fig. 2b). In tests with activated CD4<sup>+</sup> T cells, the DFT-1 mAb immunoprecipitated both the 135-kDa protein and a minor protein with lower molecular mass of 105 kDa, unlike the HSCA-2 mAb, which did not react with proteins of either of these sizes (Fig. 2b). Both the HSCA-2 and DFT-1 mAbs appeared to specifically immunoprecipitate several common, low molecular mass (25- to 40-kDa) proteins in activated CD4<sup>+</sup> T cells (data not shown).

Expression characteristics of CD43 in CD4<sup>+</sup> memory T cells

As shown in Fig. 4 (left), the CD4<sup>+</sup>CD45RO<sup>+</sup> cell population can be divided into three distinct subsets (M1, M2, and M3) on the basis of their CD43(HSCA-2) expression levels; this confirms our previous findings (31). We therefore tried to define the same three subsets on the basis of their CD43(DFT-1) expression levels (Fig. 4, right); interestingly, the proportions of the M1 subset detected by the DFT-1 mAb was significantly larger when defined by HSCA-2 mAb, and the proportion of the M3 subset defined by the DFT-1 mAb was significantly smaller than when defined by HSCA-2 mAb. We observed similar subset percentages when the 1G10 and L10 mAbs were used in place of the DFT-1 mAb (Table I). These results indicate that the low levels of CD3(HSCA-2) expression that typify the M3 population do not affect the ability of M3 cells to express other CD43 epitopes.

Accessory functions of CD43 in recall responses of CD4<sup>+</sup> memory T cells

To analyze possible accessory functions of CD43(HSCA-2) in memory T cells, we first examined the effects of exposure to HSCA-2 mAb on the recall Ag-induced proliferation of total PBMCs in culture. As shown in Fig. 5, HSCA-2 mAb seemed to

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Table I. CD4<sup>+</sup> memory T cell subsets defined by four different CD43 mAbs

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<thead>
<tr>
<th>CD43 mAb</th>
<th>M1 (%)</th>
<th>M2 (%)</th>
<th>M3 (%)</th>
</tr>
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<tbody>
<tr>
<td>HSCA-2</td>
<td>19.9 ± 4.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.8 ± 8.0</td>
<td>5.7 ± 3.0</td>
</tr>
<tr>
<td>DFT-1</td>
<td>19.0 ± 3.7</td>
<td>25.3 ± 9.4&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.0 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>L10</td>
<td>20.5 ± 4.3</td>
<td>23.6 ± 8.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.6 ± 1.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>IG10</td>
<td>19.0 ± 4.1</td>
<td>24.5 ± 8.2&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.8 ± 1.8&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup> The percentage of each subset in total CD4<sup>+</sup> T cells was determined by three-color flow cytometry, as shown in Fig. 4A.

<sup>b</sup> Average ± SD (n = 6)

Value was significantly larger or smaller than that of HSCA-2 mAb by Wilcoxon signed rank test (p < 0.05).
polyclonal T cell responses to plastic- or monocyte-bound CD3 was the CD28.2 clone, which is capable of strongly costimulating (Fig. 6). The CD28 mAb that we chose to use in this experiment /H9262 line) or without (broken line) PPD (5 \( \mu \text{g/ml} \)) in the presence of autologous CD14 \( \text{B} \), Dose responses of CD43 mAbs. PBMCs were stimulated with (solid line) or without (broken line) PPD (5 \( \mu \text{g/ml} \)) in the presence of HSCA-2 (\( \bullet \)), DFT-1 (\( \triangle \)), and MOPC21 (\( \square \)) at the various concentrations. Proliferation was measured on day 5 by adding \([^{3}\text{H}]\text{thymidine}\) during the last 16 h of culture. Results are representative of five donors.

dose-dependently accelerate the proliferation of PPD Ag-stimulated PBMCs, but had no comparable effect on their proliferation in the absence of PPD. The three traditionally used anti-CD43 mAbs (DFT-1 (Fig. 5\( \text{B} \)), and 1G10 and L10 (data not shown)) also proved at least as effective as the HSCA-2 mAb in accelerating PPD-stimulated proliferation of PBMCs. As the majority of PPD-reactive cells appear to be CD4\(^{+}\) T cells (data not shown), we examined the effects of the addition of a combination of HSCA-2 mAb and CD28 mAb on the responses of MACS-purified CD4\(^{+}\) T cells in the presence of autologous monocytes (Fig. 6). The CD28 mAb that we chose to use in this experiment was the CD28.2 clone, which is capable of strongly costimulating polyclonal T cell responses to plastic- or monocyte-bound CD3 mAb, but (if anything) inhibits Ag-specific responses (39). Thus, whereas HSCA-2 mAb led to a significantly enhanced PPD response in CD4\(^{+}\) T cells, the addition of CD28 mAb led to it being slightly inhibited. We also examined the effect of the HSCA-2 mAb on the TT-dependent proliferative response of CD4\(^{+}\) T cells and found that it had an enhancing effect (Fig. 6).

Next we examined the effects of the addition of CD28 and HSCA-2 mAbs on recall responses in each of the three CD4\(^{+}\) memory T cell subsets as defined by their separation in a cell sorter on the basis of their CD43(HSCA-2) expression levels (Fig. 4). As shown in Fig. 7A, only the M1 subset cells appeared to be capable of responding to PPD; this confirms our previous findings (31). The results of our experiments with the M1 subset mirrored our findings with unseparated CD4\(^{+}\) T cell populations, in that the PPD response of M1 subset cells was significantly and dose-dependently enhanced by the addition of HSCA-2 mAb (Fig. 7, B and C), but was inhibited, rather than enhanced, in the presence of the CD28 mAb (Fig. 7A). The PPD responses of the M2 and M3 subset cells were virtually unaffected by the addition of the HSCA-2 mAb (Fig. 7, B and C). This did not surprise us, given that the M2 and M3 subsets appeared to contain a relatively very
Synergistic effects between CD43 and CD28 mAbs in polyclonal response

To determine whether CD43(HSCA-2) can play an accessory role in the polyclonal activation of T cells, we examined the possible effects of the HSCA-2 mAb on the proliferative response of CD4+ T cells to monocyte-bound CD3 mAb (Fig. 8). We found that the HSCA-2 mAb did have an effect, but that it was only marginally costimulatory at lower (0.001–0.1 μg/ml) CD3 mAb concentrations and that its effectiveness disappeared at the highest concentration tested (1 μg/ml). Interestingly, the results shown in Fig. 8A provide convincing evidence that the CD28/HSCA-2 mAb combination had a synergistic effect on the CD3 mAb-mediated polyclonal response (Fig. 8A). A similar synergistic effect on the CD3-mediated response was observed when the mAbs involved were DFT-1 and CD28 (data not shown).

Cells of two of the three CD4+ memory T cell subsets (M1 and M2) responded strongly to monocyte-bound CD3 mAb, whereas M3 subset cells did not (Fig. 8B). These findings are in agreement with those in our original report (31). The HSCA-2 mAb was almost as effective as CD28 mAb in their enhancement of polyclonal responses in M1 and M2 subset cells, but had no such effect in M3 subset cells. There were marginal synergistic effects on polyclonal responses when M1 and M2 subset cells were cotreated with the HSCA-2 and CD28 mAbs, although a much more obvious synergistic effect became evident when we used RO− naive subset cells instead.

When the Fab portion of the HSCA-2 mAb was used instead of intact Ab, we could see no indication of either an enhanced PPD-mediated stimulatory response or a synergistic interaction involving the CD28 mAb and CD3 mAb polyclonal responses (data not shown).

Discussion

HSCA-2 mAb specifically recognizes a neuraminidase-sensitive epitope on the low molecular mass (115-kDa) glycoform of the CD43 molecule that is predominantly expressed in lymphoid cells, including resting T and NK cells. By contrast, all previously described CD43 mAbs (including the DFT-1 mAb) react strongly or even very strongly with a larger (135-kDa) CD43 glycoform that is expressed in myeloid cells such as monocytes and granulocytes. Importantly, the HSCA-2 mAb does not appear to recognize the 135-kDa glycoform and hence binds only marginally, if at all, to myeloid cells; it also does not immunoprecipitate a third high molecular mass (125-kDa) CD43 protein that is recognized by the DFT-1 mAb in both KG-1 and CD4+ T cells. Taken together, these findings suggest that the HSCA-2 mAb is specific for a novel glycoepitope on the 115-kDa glycoform of CD43.

Interestingly, HSCA-2 mAb differs from all pre-existing CD43 mAbs in being unable to recognize the high molecular mass CD43 glycoform (135 kDa) that is present on activated CD4+ T cells. Thus, the 135-kDa CD43 glycoform consists of a more fully glycosylated version that is generated in the course of the increase in molecular mass of the 115-kDa CD43 glycoform that occurs during T cell activation (5, 7); it is possible that the HSCA-2 glycoepitope is either lost or masked in the course of this glycosyl modification process. As the molecular mass of the CD43 polypeptide is ∼40 kDa, the 25- to 40-kDa proteins recognized by HSCA-2 mAb in activated T cells could well be degradation forms of CD43, and we have even observed what appeared to be the gradual disappearance of CD43(HSCA-2) epitopes from a subpopulation of activated CD4+CD45RO+ T cells (manuscript in preparation).

We noticed that there were significant differences in the distribution of the CD4 memory T cell subsets depending upon which of the available CD43 mAbs was used in their separation. Thus, for example, the percentages of M2 subset cells that we observed in separations achieved using the HSCA-2 mAb were significantly smaller than those observed in separations using any of the other available mAbs. In the case of the M3 subsets, the percentages were larger with the HSCA-2 mAb than with any of the others. These CD43 mAb-dependent differences in subset distributions...
may correspond to differences in such subset cell functions as memory vs anergy, but we have yet to explore this possibility in any detail.

In this report we show that HSCA-2 and certain other CD43 mAbs are capable of accelerating both the recall Ag-induced and CD3 mAb-induced proliferation of CD4 memory T cells. There are previous reports indicating that CD43 molecules may also have accessory involvements in T cell activation, such as, for example, in mice, where CD43 mAb appears to costimulate T cell activation during treatment with plastic-bound CD3 mAb and alloantigens (17, 40). In humans, however, there does not appear to be any evidence of CD43 mAb being involved in a costimulatory capacity in the polyclonal activation of T cells (41). In situations involving Ag-specific responses, there is one report that CD43 is necessary for the production of IL-2 in HLA class II-specific human hybridoma T cells (42), but it is important to note that the experimental system used to obtain these data was an unusually artificial one. There are, however, several publications in which it is claimed that a number of CD43 mAbs can stimulate Ag-independent human T cell proliferation in a multicomponent test system that requires the presence of CD43-stimulated monocytes (43–46). Thus, the results described in this study appear to provide the first evidence that T cell–determined CD43 may help to stimulate the Ag-specific proliferative responses of freshly isolated T cells in humans. In the case of our new mAb (HSCA-2), we can exclude any involvement of CD43-mediated monocyte stimulation in T cell activation. The reason why HSCA-2 mAb differs from all previously used CD43 mAbs in this important way is that it appears to lack reactivity to the 135-kDa CD43 glycoform expressed in monocytes.

Given the above consideration, it seems reasonable to assume that CD43 plays a part in some of the cell signaling events that are likely to be involved in memory T cell activation. Up-regulated expression of CD43 in M1 subset cells may cause an increase in activation signaling in concert with other up-regulated costimulatory molecules such as CD28 (31). Our observation that CD43 and CD28 mAbs act synergistically to stimulate the polyclonal response of CD4+ T cells to anti-CD3 mAb may indicate that both CD43 and CD28 have an accessory signaling role in the induction of the polyclonal response. It has previously been reported that T cell activation through CD43 cross-linking in humans induces serine phosphorylation of Cbl proteins and tyrosine phosphorylation of Vav (19, 20). It has also been reported that one of the CD28–determined costimulatory signaling processes is mediated by tyrosine phosphorylation of Vav1, which is, in turn, negatively regulated by Cbl-b (47–49). Thus, although the precise molecular mechanisms underlying the costimulatory effects of CD43 remain to be determined, it is possible that both CD28 and CD43 are capable of synergistically enhancing the activation of CD4+ memory T cells by a mechanism involving the common signaling pathway.

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