Two Distinct T Cell Subsets, CD4+ and CD8+ CD60+, and Their Cytokines Are Required for In Vitro Induction of Human Ragweed-Specific Memory IgE Responses

Tamar A. Smith-Norowitz, Jonathan Silverberg, Kevin B. Norowitz, Martin H. Bluth, Seto Chice, Rauno Joks, Maja Nowakowski and Helen G. Durkin

*J Immunol* 2008; 181:4761-4769;

doi: 10.4049/jimmunol.181.7.4761

http://www.jimmunol.org/content/181/7/4761

**References**

This article cites 29 articles, 10 of which you can access for free at:

http://www.jimmunol.org/content/181/7/4761.full#ref-list-1

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at:

http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at:

http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:

http://jimmunol.org/alerts
Two Distinct T Cell Subsets, CD4$^{+}$ and CD8$^{+}$CD60$^{+}$, and Their Cytokines Are Required for In Vitro Induction of Human Ragweed-Specific Memory IgE Responses

Tamar A. Smith-Norowitz,† Jonathan Silverberg,‡ Kevin B. Norowitz,† Martin H. Bluth,* Seto Chice,† Rauno Joks,* Maja Nowakowski,**† and Helen G. Durkin*‡

CD8$^{+}$CD60$^{+}$ T cells (80–98% CD45RO$^{+}$; 20% CD23$^{+}$) are significantly increased in the blood of serum IgE$^{+}$ ragweed-sensitized (RS) compared with serum IgE-nonatopic humans ($p = 0.001$). CD8$^{+}$CD60$^{+}$ T cells of the RS patients produced IL-2, IL-4, IL-10, IL-12, IFN-α, and IFN-γ, but not IL-6 or IL-13. When their PBMC were cultured with ragweed Ag (RA), peak IgE responses occurred on day 10; none was induced with non-cross-reacting or without Ag; nonatopic PBMC did not respond to any stimulant. When either CD4$^{+}$ or CD8$^{+}$CD60$^{+}$ T cells were depleted from RS PBMC before culture with RA, no IgE responses were induced. If purified CD4$^{+}$ T cells or low numbers of CD8$^{+}$CD60$^{+}$ T cells were added back to the depleted PBMC, IgE responses were restored. However, higher numbers of CD8$^{+}$CD60$^{+}$ T cells totally suppressed IgE responses. Total suppression also was obtained when RS PBMC were cultured with RA and either anti-IL-2, IL-4, IL-10, IL-12, IFN-γ (all concentrations), or IFN-α (low concentrations), but not anti-IL-6 or IL-13. Higher concentrations of anti-IFN-α potentiated IgE responses. *The Journal of Immunology, 2008, 181: 4761–4769.

It is well recognized that CD4$^{+}$ T cells and their cytokines (IL-4 and IL-13) are required for induction of human and murine IgE responses in vivo and in vitro (reviewed in Refs. 1 and 2). However, there is evidence that different cell/cytokine pathways are required for induction/maintenance of memory IgE responses. Herrick et al. (3) were the first to demonstrate that in pathways are required for induction/maintenance of memory IgE responses by spleen cells of BPO-keyhole limpet hemocyanin (KLH)-sensitized mice required two distinct T cell subsets: Thy 1$^{+}$ asialo GM1 ganglioside-negative and Thy 1$^{+}$ asialo GM1 ganglioside-positive T cells (3); in her studies, IL-4 was responsible for approximately one-half of the memory IgE response and other cytokines (IFN-α and IFN-γ) for the remainder of the memory IgE response. Studies of Auci et al. (4), in the same BPO-KLH-sensitized mice showed that CD8$^{+}$ T cells totally suppressed IgE responses. Total suppression also was obtained when RS PBMC were cultured with RA and either anti-IL-2, IL-4, IL-10, IL-12, IFN-γ (all concentrations), or IFN-α (low concentrations), but not anti-IL-6 or IL-13. Higher concentrations of anti-IFN-α potentiated IgE responses.

Smith-Norowitz (6) and Smith-Norowitz et al. (7) found that there is a statistically significant increase in numbers of the CD8$^{+}$CD60$^{+}$ T cells (CD60 also is a ganglioside) in the blood of serum IgE$^{+}$ ragweed-sensitized (RS) humans at the peak of the ragweed allergy season. Others demonstrated that CD8$^{+}$ T cells, like CD4$^{+}$ T cells, help IgG responses (8, 9) and that CD60$^{+}$ T cells also help these responses (10). However, the role of CD8$^{+}$ or CD60$^{+}$ T cells in IgE responses and T cells that simultaneously express both CD8 and CD60 in humoral responses, including IgE responses, has not been studied. The present studies further characterize CD8$^{+}$CD60$^{+}$ T cells and other lymphocytes in the blood of RS humans obtained at the peak of the ragweed allergy season and investigate the ability of CD8$^{+}$CD60$^{+}$ T cells to regulate human memory IgE responses.

We found that CD8$^{+}$CD60$^{+}$CD45RO$^{+}$ T cell numbers are greatly increased in the blood of serum IgE$^{+}$ RS humans at the peak of the ragweed allergy season. CD8$^{+}$CD60$^{+}$ T cells and their cytokines (IL-2, IL-4, IL-10, IL-12, IFN-α, and IFN-γ) are required for induction of human ragweed-specific memory IgE responses; CD4$^{+}$ T cells also are required for memory IgE responses, as previously shown by others (reviewed in Refs. 1 and 2). CD8$^{+}$CD60$^{+}$ T cells and IFN-α in high numbers/concentration suppressed induction of memory IgE responses.

Materials and Methods

Subjects

All subjects ($n = 32$) were medical student volunteers (males and females, ages 22–40 years). At the time of the study, none of the subjects received allergy therapy and none was being treated with any medication. RS subjects may or may not have exhibited a past history of clinical allergic reactions/symptoms. Approval was obtained from the State University of New York (SUNY) Downstate Institutional Review Board and the procedures followed were in accordance with institutional guidelines involving human subjects.

Peripheral blood (40 ml) was obtained from subjects 2 days after they were defined as either RS, serum IgE$^{+}$ ($>100$ IU/ml; $n = 20$), or nonatopic (NA) serum IgE$^{+}$ (<100 IU/ml; $n = 12$). In August–September, the peak of the ragweed allergy season in New York City. Subjects were skin tested by intradermal injection of pollen Ags (mixed ragweed (tall and short);
T CELL/CYTOKINE NETWORKS IN MEMORY IgE RESPONSES

Dulbecco’s PBS (D-PBS; 4 ml; Life Technologies) was added to the tubes, which were then centrifuged at 300 × g for 5 min at room temperature. Supernatants were discarded, cells were resuspended in Intraprep Reagent 2 (100 µl; Beckman Coulter), and the tubes were incubated for 5 min at room temperature. Tubes were then gently agitated, after which Abs to individual cytokines (20 µl; BD Pharmingen) were added: PE-conjugated mouse anti-human IL-2, IL-4, or IL-12; PE-conjugated rat-anti-human IL-6, IL-10, or IL-13; and PE-conjugated mouse anti-human IFN-γ or IFN-α. The tubes were incubated for 10 min at room temperature, after which D-PBS (4 ml) was added, and the tubes were centrifuged at 300 × g for 5 min at room temperature. Supernatants were discarded, pellets were resuspended in D-PBS (400 µl) and D-PBS with 0.1% paraformaldehyde (100 µl, ImmuNoPrep C; Beckman Coulter). Cells were then counted with a Coulter Epics XL/MCL Flow Cytometer using System II software (Coulter), with CytoComp (Coulter) and QC Windows (Flow Cytometry Systems) used to ensure consistent instrument settings. Data are expressed as percent total lymphocytes. Some experiments were conducted using PBMC from Ficol-Paque gradients, with similar results obtained.

Confocal microscopy: immunofluorescence labeling

Human PBMC (1 × 10^6/ml) were washed once with D-PBS, then fixed with 4% paraformaldehyde (Sigma-Aldrich) for 20 min at room temperature, after which they were washed with D-PBS. The cells were then incubated with 0.1% glycine buffer (Sigma-Aldrich) for 10 min at room temperature and then washed twice in D-PBS. Cells were then resuspended in D-PBS (1.0 × 10^6/ml). For all cell-labeling experiments, normal goat serum (NGS) incubation steps were for 15 min and Ab incubations were for 15 min at room temperature, followed by two washing steps with D-PBS.

To label CD8^+^CD60^+^ TCRαβ^+^ T cells, PBMC were incubated in the following sequence: NGS: primary Ab (mouse IgG anti-human CD8 (2–5 µg/µl), clone G42–8; BD Pharmingen) and secondary Ab (Alexa Fluor 647 goat anti-rat IgG, 1/200 dilution in 0.1% BSA; Molecular Probes/Invitrogen); NGS: primary Ab (mouse IgM mAb anti-human CD60 (2–5 µg/µl, clone UM4D4; Ancell) and secondary Ab (Alexa Fluor 488 goat anti-mouse IgM, 1/200 dilution in 0.1% BSA; Molecular Probes); and NGS: primary Ab (mouse IgM anti-human TCRαβ (2–5 µg/µl, clone T1089.1A-31; BD Pharmingen) and secondary Ab (Alexa Fluor 568 goat anti-mouse IgM, 1/200 dilution in 0.1% BSA; Molecular Probes).

Peripheral blood (3 ml) was added to RPMI 1640 (1:1 ratio containing 2-glutamine (2 mM), penicillin (100 U/ml), and streptomycin (100 µg/ml, t-glutamine-penicillin-streptomycin solution; Sigma-Aldrich). Phychotrine, magglutinin P (60 µl/ml; Difco Laboratories) and GolgiPlug (1 µl/ml; BD Pharmingen) were added to the blood, which was then incubated for 6 h at 37°C. Blood (50 µl) was then incubated with specific mAb (10 µl): CD8-PC5 (IOT; Beckman Coulter) or CD4-PC5 (IOT; Beckman Coulter) plus CD45RO-EC5D (IOT; Beckman Coulter) plus CD60-FTC (Ancell) for 10 min at room temperature in the dark, after which Intraprep Reagent 1 (100 µl, Intraprep Kit; Beckman Coulter ImmunoCham) was added to each tube and the tubes were vortexed and then incubated for 15 min at room temperature in the dark according to the manufacturer’s recommendation.

Flow cytometry

For flow cytometry and cell culture studies, blood was collected into EDTA Monocyte tubes (Sherwood Medical) and retained for up to 2 h at room temperature. PBMC used in cell cultures were separated from whole blood as percent CD8^+^ T cells. Some experiments were conducted using PBMC from Ficol-Paque gradients, with similar results obtained.

Confocal microscopy: immunofluorescence labeling

Human PBMC (1 × 10^6/ml) were washed once with D-PBS, then fixed with 4% paraformaldehyde (Sigma-Aldrich) for 20 min at room temperature, after which they were washed with D-PBS. The cells were then incubated with 0.1% glycine buffer (Sigma-Aldrich) for 10 min at room temperature and then washed twice in D-PBS. Cells were then resuspended in D-PBS (1.0 × 10^6/ml). For all cell-labeling experiments, normal goat serum (NGS) incubation steps were for 15 min and Ab incubations were for 15 min at room temperature, followed by two washing steps with D-PBS.

To label CD8^+^CD60^+^ TCRαβ^+^ T cells, PBMC were incubated in the following sequence: NGS: primary Ab (mouse IgG anti-human CD8 (2–5 µg/µl), clone G42–8; BD Pharmingen) and secondary Ab (Alexa Fluor 647 goat anti-rat IgG, 1/200 dilution in 0.1% BSA; Molecular Probes/Invitrogen); NGS: primary Ab (mouse IgM mAb anti-human CD60 (2–5 µg/µl, clone UM4D4; Ancell) and secondary Ab (Alexa Fluor 488 goat anti-mouse IgM, 1/200 dilution in 0.1% BSA; Molecular Probes); and NGS: primary Ab (mouse IgM anti-human TCRαβ (2–5 µg/µl, clone T1089.1A-31; BD Pharmingen) and secondary Ab (Alexa Fluor 568 goat anti-mouse IgM, 1/200 dilution in 0.1% BSA; Molecular Probes).

Cell cultures

PBMC depleted or not of CD4^+^ or CD8^+^ T cells (2 × 10^6/ml in complete medium) were placed in 12 × 75-mm (5 ml) polystyrene tubes (Fisher Scientific) with or without purified ragweed Ag (1–100 µl, tall and short ragweed pollens; Center Laboratories), with or without OVA (1–100 ng), and cultured for 0–12 days at 37°C in a humidified atmosphere of 4% CO2 in air. Tubes were centrifuged at 700 × g for 10 min at room temperature, supernatants collected, and stored at −20°C until assayed for IgE. The concentration of IgE in supernatants was determined by using a competitive phase sandwich ELISA (Total IgE Microplate Test Kits; Kallestead Diagnostics or HOPE) performed according to the manufacturers’ recommen-
dations. Specimens were analyzed in duplicate and a standard curve was derived from known concentrations of IgE. Plates were read using an automated microplate reader (model Elx800; Bio-Tek Instruments), with a minimum measurement from each reference filter (Flow-Tech Instruments). OD values were converted to IU/ml and ng/ml (1 IU/ml = 2.4 ng/ml). Complete RPMI 1640 medium (Fisher Scientific) consisted of HEPES (2.5 mM/100 ml; Fisher Scientific), penicillin (100 U/ml; Life Technologies),
Depletion of CD4+ CD8+ T cells and CD8+ CD60+ T cells

PBMC were first depleted of CD14+ (CD4−/CD8−) monocytes by incubating PBMC (1 × 10^6/ml) with anti-human CD14 mAb-coated magnetic beads (25 μl of beads/ml of cells) (Dynabeads CD14; Invitrogen/Dynal) in 5-ml tubes (Fisher Scientific) for 20 min at 2–8°C, with gentle tilting and rotation, according to the manufacturer’s recommendation. Tubes were then placed on MFC-1 magnets (Invitrogen/Dynal) for 2 min to allow bead-bound cells to collect on walls. Supernatants were removed and stored on ice. Tubes were removed from the magnets and bead-bound cells were recovered, washed in buffer 1 by centrifuging at 1000 rpm for 5 min at room temperature, and resuspended in buffer 2 (1 × 10^6/ml). Bead-bound cells were detached from magnetic beads using CD4 Detachabeads (1 × 10^7/ml; Dynal), washed twice in buffer 2, and transferred to a fresh tube and cells were washed twice in buffer 2 by centrifuging at 1000 rpm for 5 min at room temperature. The pellet was resuspended in buffer 2 (1 ml) and stored at room temperature, with gentle mixing. Tubes were then placed on the magnets for 2 min to allow beads to collect on walls, after which supernatants containing released cells were transferred to a fresh tube and cells were washed twice in buffer 2 by centrifuging at 1000 rpm for 5 min at room temperature. The pellet was resuspended in buffer 2 (1 ml) and stored at room temperature.

CD4+ CD8+ T cells were depleted from PBMC which had been depleted of CD4+ T cells by incubating cells (1 × 10^7/ml) with anti-human CD4 mAb-coated magnetic beads (25 μl of beads/ml of cells) (Dynal CD4+ isolation Kit; Invitrogen/Dynal) in 5-ml tubes for 20 min at 2–8°C, with gentle tilting and rotation, according to the manufacturer’s recommendation. Tubes were then placed on MFC-1 magnets (Invitrogen/Dynal) for 2 min to allow bead-bound cells to collect on walls. Supernatants were removed and stored on ice. Tubes were removed from the magnets and bead-bound cells were recovered, washed in buffer 1 by centrifuging at 1000 rpm for 5 min at room temperature, and resuspended in buffer 2 (1 × 10^6/ml). Bead-bound cells were detached from magnetic beads using CD4 Detachabeads (1 × 10^7/ml; Dynal), washed twice in buffer 2, and resuspended in buffer 2 (1 ml) and stored at room temperature.

Depletion of CD8+ CD60+ T cells

CD8+ T cells were depleted from PBMC by incubating cells (1 × 10^7/ml) with anti-human CD8 mAb-coated magnetic beads (25 μl of beads/ml of cells) (Dynal CD8+ isolation Kit; Invitrogen/Dynal) in 5-ml tubes for 20 min at 2–8°C, with gentle tilting and rotation, according to the manufacturer’s recommendation. Tubes were then placed on MFC-1 magnets (Invitrogen/Dynal) for 2 min to allow bead-bound cells to collect on walls. Supernatants were removed and stored on ice. Tubes were removed from the magnets and bead-bound cells were recovered, washed in

---

Table I. Distributions of lymphocyte subpopulations in blood of serum IgE+ RS humans

<table>
<thead>
<tr>
<th>Subject</th>
<th>Serum IgE (IU/ml)</th>
<th>Skin Test</th>
<th>Fluorescent Cells (mm^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CD3^+</td>
</tr>
<tr>
<td>1</td>
<td>&gt;1000</td>
<td>+</td>
<td>1787</td>
</tr>
<tr>
<td>2</td>
<td>301</td>
<td>+</td>
<td>1654</td>
</tr>
<tr>
<td>3</td>
<td>230</td>
<td>+</td>
<td>994</td>
</tr>
<tr>
<td>4</td>
<td>123</td>
<td>+</td>
<td>888</td>
</tr>
<tr>
<td>5</td>
<td>110</td>
<td>+</td>
<td>1660</td>
</tr>
<tr>
<td>6</td>
<td>105</td>
<td>+</td>
<td>1446</td>
</tr>
<tr>
<td>7</td>
<td>320</td>
<td>+</td>
<td>1851</td>
</tr>
<tr>
<td>8</td>
<td>229</td>
<td>+</td>
<td>782</td>
</tr>
<tr>
<td>9</td>
<td>377</td>
<td>+</td>
<td>1928</td>
</tr>
<tr>
<td>10</td>
<td>182</td>
<td>+</td>
<td>1311</td>
</tr>
<tr>
<td>11</td>
<td>525</td>
<td>+</td>
<td>1862</td>
</tr>
<tr>
<td>12</td>
<td>578</td>
<td>+</td>
<td>1183</td>
</tr>
<tr>
<td>13</td>
<td>181</td>
<td>+</td>
<td>1286</td>
</tr>
<tr>
<td>14</td>
<td>206</td>
<td>+</td>
<td>1863</td>
</tr>
<tr>
<td>15</td>
<td>344</td>
<td>+</td>
<td>1435</td>
</tr>
<tr>
<td>16</td>
<td>732</td>
<td>+</td>
<td>1044</td>
</tr>
<tr>
<td>17</td>
<td>107</td>
<td>+</td>
<td>2134</td>
</tr>
<tr>
<td>18</td>
<td>&gt;1000</td>
<td>+</td>
<td>952</td>
</tr>
<tr>
<td>19</td>
<td>&gt;1000</td>
<td>+</td>
<td>1199</td>
</tr>
<tr>
<td>20</td>
<td>216</td>
<td>+</td>
<td>1346</td>
</tr>
</tbody>
</table>

---

a Flow cytometry data are expressed as total cells/mm^3.

b 1 IU = 2.4 ng.

c Serum IgE+ RS humans allergic to standardized pollen, mite, or cat Ags.

d nt. Not tested.
buffer 1 by centrifuging at 1000 rpm for 5 min at room temperature, and resuspended in buffer 2 (1 × 10^7/ml). CD8⁺ bead-bound cells were detached from the magnetic beads using CD8 Detachabeads (1 × 10^7 cells/25 μl of Detachabeads; Dynal), with cells incubated for 45 min at room temperature, with gentle mixing. Tubes were then placed on the magnet for 2 min to allow beads to collect on walls, after which supernatants containing released cells were transferred to a fresh tube and cells were washed twice in buffer 2 by centrifuging at 1000 rpm for 5 min at room temperature. The pellet was resuspended in buffer 2 (1 ml) and stored at room temperature. If >1% CD8⁺ CD3⁺ T cells were detected in supernatants, the separation procedure was repeated. Purity of CD8⁺ (CD60⁺/-) T cells recovered from beads was >99% (flow cytometry).

To separate CD8⁺ CD60⁺ T cells from CD8⁺ CD60⁻ T cells, CD8⁺ T cells (1 × 10^7/ml) were incubated with mouse IgM anti-CD60 mAb (80 μl of titrated anti-CD60; Ancell) in 5-ml tubes for 20 min at 2–8°C, with gentle tilting and rotation. Cells were washed twice in PBS (2–3 ml) by centrifuging at 1000 rpm for 5 min at room temperature, after which they were resuspended in buffer 1 (1.0 × 10^7/ml). Rat anti-mouse IgM Dynabeads (25 μl/ml; Invitrogen/Dynal) were added to cells, which were then incubated for 10 min at 2–8°C, with gentle tilting and rotation, according to the manufacturer’s recommendation. Tubes were then placed on magnets for 2 min to allow bead-bound cells to collect on walls, after which supernatants were recovered and stored on ice. Bead-bound cells were recovered, washed three to four times in buffer 1 by centrifuging at 1000 rpm for 5 min at room temperature, resuspended in buffer 2 (1 × 10^7/ml), and incubated in complete medium for up to 18 h at 37°C, when cells had dissociated from beads. Tubes were placed on magnets for 2 min to allow beads to collect on walls and the cell-containing supernatants were recovered and stored at room temperature. Purity of CD8⁺ CD60⁺ (CD3⁺) T cells was >99% (flow cytometry).

Reconstitution of PBMC with purified CD8⁻ CD60⁻ T cells and varying numbers of purified CD8⁺ CD60⁺ (CD3⁺) T cells

After depletion of CD8⁺ CD60⁻ (CD3⁺) T cells from PBMC, purified CD8⁺ CD60⁺ T cells were added back to PBMC depleted of CD8⁺ T cells in the same ratio as was present in each subject’s PBMC before fractionation. Purified CD8⁺ CD60⁺ (CD3⁺) T cells, in varying numbers (0.01–10.0 × 10^6), were then added back to the CD8⁻ CD60⁻ T cell-depleted PBMC (2 × 10^7) that had been reconstituted with CD8⁺ CD60⁺ T cells and cells were cultured as described. In some experiments, purified CD8⁻ CD60⁻ T cells (0.01–10.0 × 10^7) were added to unfractionated PBMC (2 × 10^7) before culture.

Statistical analysis

Lymphocyte distributions and serum IgE levels of serum IgE⁻ and serum IgE⁺ subjects were compared on each variable. Significance between variables was determined using Student’s t test. A value of p < 0.05 was considered statistically significant for all comparisons. The degree of association between these measures was assessed using Pearson’s correlations. Statistical analyses were performed using SPSS for Windows, version 10.0 software.

---

Table II. Distributions of lymphocyte subpopulations in blood of serum IgE⁻ humans

<table>
<thead>
<tr>
<th>Subject</th>
<th>Serum IgE (IU/ml)</th>
<th>Skin Test</th>
<th>CD3⁺</th>
<th>CD4⁺</th>
<th>CD8⁺</th>
<th>CD8⁻ CD60⁺</th>
<th>CD8⁺ CD60⁻</th>
<th>TCRαβ⁺</th>
<th>TCRγδ⁺</th>
<th>CD19⁺</th>
<th>CD16/56⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76</td>
<td>–</td>
<td>704</td>
<td>463</td>
<td>370</td>
<td>342</td>
<td>28</td>
<td>527</td>
<td>302</td>
<td>434</td>
<td>274</td>
</tr>
<tr>
<td>2</td>
<td>59</td>
<td>–</td>
<td>1575</td>
<td>926</td>
<td>665</td>
<td>657</td>
<td>8</td>
<td>nt⁺</td>
<td>nt⁺</td>
<td>213</td>
<td>523</td>
</tr>
<tr>
<td>3</td>
<td>21</td>
<td>–</td>
<td>1097</td>
<td>717</td>
<td>478</td>
<td>422</td>
<td>56</td>
<td>81</td>
<td>121</td>
<td>476</td>
<td>234</td>
</tr>
<tr>
<td>4</td>
<td>19</td>
<td>–</td>
<td>1028</td>
<td>615</td>
<td>430</td>
<td>426</td>
<td>4</td>
<td>1035</td>
<td>344</td>
<td>405</td>
<td>272</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>–</td>
<td>698</td>
<td>548</td>
<td>126</td>
<td>97</td>
<td>29</td>
<td>867</td>
<td>246</td>
<td>420</td>
<td>263</td>
</tr>
<tr>
<td>6</td>
<td>87</td>
<td>–</td>
<td>1108</td>
<td>759</td>
<td>278</td>
<td>249</td>
<td>29</td>
<td>1348</td>
<td>245</td>
<td>672</td>
<td>515</td>
</tr>
<tr>
<td>7</td>
<td>72</td>
<td>–</td>
<td>1386</td>
<td>656</td>
<td>640</td>
<td>570</td>
<td>70</td>
<td>1200</td>
<td>69</td>
<td>222</td>
<td>162</td>
</tr>
<tr>
<td>8</td>
<td>18</td>
<td>–</td>
<td>1092</td>
<td>626</td>
<td>422</td>
<td>352</td>
<td>74</td>
<td>1034</td>
<td>73</td>
<td>116</td>
<td>189</td>
</tr>
<tr>
<td>9</td>
<td>19</td>
<td>–</td>
<td>1035</td>
<td>705</td>
<td>255</td>
<td>181</td>
<td>74</td>
<td>1005</td>
<td>75</td>
<td>195</td>
<td>210</td>
</tr>
<tr>
<td>10</td>
<td>42</td>
<td>–</td>
<td>291</td>
<td>357</td>
<td>92</td>
<td>62</td>
<td>30</td>
<td>478</td>
<td>10</td>
<td>284</td>
<td>136</td>
</tr>
<tr>
<td>11</td>
<td>31</td>
<td>–</td>
<td>411</td>
<td>238</td>
<td>137</td>
<td>101</td>
<td>36</td>
<td>387</td>
<td>60</td>
<td>86</td>
<td>70</td>
</tr>
<tr>
<td>12</td>
<td>45</td>
<td>–</td>
<td>1052</td>
<td>802</td>
<td>322</td>
<td>293</td>
<td>39</td>
<td>1115</td>
<td>274</td>
<td>235</td>
<td>430</td>
</tr>
</tbody>
</table>

*Flow cytometry data are expressed as total cells/mm³.

Standard conversion: 1 IU = 2.4 ng.

nt, Not tested.
Results
Serum IgE+ RS and serum IgE− NA subjects
All subjects defined as RS (n = 20) were skin test positive to ragweed Ag and to other allergens tested (see Materials and Methods) 2 days before their blood was obtained for the present studies, and all were serum IgE+ (107 to >1000 IU/ml; Table I). Subjects defined as NA (n = 12) were skin test negative to ragweed Ag and to the other allergens; all NA subjects were serum IgE− (<100 IU/ml; Table II). Serum IgE levels were significantly increased in RS compared with NA subjects (407 ± 76 and 42 ± 7, respectively; p = 0.001; individual data shown in Tables I and II).

Lymphocyte distributions. The distributions of blood T cells (CD3+, CD4+, CD8+, TCRαβ+) dramatically increased in RS compared with NA subjects (CD3: 1430/mm³ ± 88 and 956 ± 107, respectively; p = 0.002; CD4: 834/mm³ ± 52 and 618 ± 56, respectively; p = 0.008; CD8: 555/mm³ ± 50 and 352 ± 54, respectively; p = 0.01; TCRαβ: 1334/mm³ ± 81 and 887 ± 109, respectively; p = 0.003; Fig. 1 and Tables I and II). The increase in CD8+ T cells in RS, compared with NA subjects, included a dramatic increase in CD8+ T cells that simultaneously expressed CD60 (>2-fold) (107/mm³ ± 14 and 39 ± 7.0, respectively; p = 0.001). Similar numbers of CD8+CD60+ T cells were detected in both groups (p > 0.05). CD4+CD60+ T cells were not studied. In contrast, the distributions of TCRyΔ+ T cells, CD19+ B cells, and CD16/56+ NK precursor cells were similar in the blood of RS and NA subjects (p > 0.05). No CD1d+ T cells were detected in the blood of either RS or NA subjects (<1%; data not shown in Fig. 1).

Although the numbers of CD8+CD60+ T cells and serum IgE levels were significantly increased in RS compared with NA subjects, the correlation between the numbers of CD8+CD60+ T cells and serum IgE levels was not significant (p > 0.40; see Table I).

CD8+CD60+ T cell subsets. CD8+CD60+ T cells in the blood of RS and NA subjects were virtually all TCRαβ+ (>98%; Figs. 2 and 3). The numbers of CD8+CD60+ T cells expressing the memory marker CD45RO were dramatically increased in the RS, compared with NA, subjects (80–98%, ~15%, respectively; see also Fig. 3). A subset of the CD8+CD60+ T cells of RS, but not NA subjects, expressed CD23 (~20%).
Seasonal changes in CD8<sup>+</sup>CD60<sup>+</sup> T cell numbers in the blood of a RS human. In the present studies, excluding those described in this section, blood of RS and NA subjects always was obtained at the peak of the ragweed allergy season. Fig. 4 shows data for blood from an extensively studied RS subject over a period of 25 mo, including ragweed allergy season and outside ragweed allergy season. CD8<sup>+</sup>CD60<sup>+</sup> T cells were present in high numbers in August or September, the peak of the ragweed allergy season. However, excluding January 2006, the numbers of CD8<sup>+</sup>CD60<sup>+</sup> blood T cells were greatly decreased at other times of the year. There was little change in serum IgE during the entire study period, and the numbers of blood CD8<sup>+</sup>CD60<sup>+</sup>T cells did not correlate with total serum IgE levels.

In vitro induction of ragweed-specific memory IgE responses

When PBMC (2 × 10<sup>6</sup>) were cultured for 0–12 days with ragweed Ag, IgE was first detected on day 5, reached peak levels on day 10, and decreased on day 12 (Fig. 5). No IgE responses were induced with non-cross-reacting Ag (OVA) or without Ag. PBMC of NA humans never produced IgE in response to any stimulant. In subsequent experiments, IgE levels were determined on day 10 of culture with ragweed Ag.

Role of CD8<sup>+</sup>CD60<sup>+</sup> and CD4<sup>+</sup> T cells in induction of memory IgE responses

Depletion and reconstitution studies (see Materials and Methods) established that both CD8<sup>+</sup>CD60<sup>+</sup> T cells and CD4<sup>+</sup> T cells are required for in vitro induction of ragweed-specific memory IgE responses by PBMC of RS humans. CD8<sup>+</sup>CD60<sup>+</sup> T cells. When PBMC of RS humans were depleted of CD8<sup>+</sup>CD60<sup>+</sup> T cells before culture with ragweed Ag, the IgE responses observed with unfractionated PBMC (Figs. 5 and 6A) were not induced on days 0–12 of culture (data for day 10 shown in Fig. 6A). When low numbers of purified...
same human were cultured for 10 days with ragweed Ag (10 ng/ml: IgE, 15 and 4767) and IFN-γ (all concentrations tested) or IL-4 (highest concentration only), in vitro induction of ragweed-specific memory IgE responses was abrogated. Interestingly, anti-IFN-α had a bimodal effect, in that IgE responses decreased with the lowest concentration of anti-IFN-α (1.0 U/ml), whereas an intermediate concentration (10.0 U/ml) potentiated IgE responses and the highest concentration (100 U/ml) had no effect. Anti-IL-6 and anti-IL-13 had no effect on IgE responses at any concentration tested.

Discussion

The present studies are the first to report that 1) CD8⁺CD60⁺ (CD45RO⁺) T cell numbers are significantly increased in blood of serum IgE⁺ RS humans in ragweed allergy season, a subset of which expressed CD23; 2) CD8⁺CD60⁺ T cells and cytokines made by CD8⁺CD60⁺ T cells (IL-2, IL-4, IL-10, IL-12, IFN-α, and IFN-γ) are required for induction of human memory IgE responses; 3) CD4⁺ T cells also are required for induction of these responses; and 4) CD8⁺CD60⁺ T cells and IFN-α, depending on their numbers/concentration, can either help or suppress induction of memory IgE responses.

The CD8⁺CD60⁺ T cells found in high numbers in blood of RS humans during peak ragweed allergy season were comprised of cells that coexpressed the memory marker CD45RO (80–98%) compared with ~15% in NA humans. From experiments in which purified CD8⁺CD60⁺ T cells were used to restore memory IgE responses by PBMC depleted of CD8⁺CD60⁺ T cells, it is known that CD8⁺CD60⁺ T cells are required for memory IgE responses. The organ(s) in which blood CD8⁺CD60⁺ (CD45RO⁺) T memory cells were generated is unknown. The number of CD8⁺CD60⁺ T cells with specificity for ragweed Ag also is unknown because the RS donors of these cells also were sensitized to other allergens (skin testing; see Materials and Methods). However, the CD8⁺CD60⁺ blood T cells were 1) obtained in peak ragweed allergy season and 2) required for induction of ragweed-specific memory IgE responses. Therefore, many, if not most, of the CD8⁺CD60⁺CD45RO⁺ T cells probably are associated with specificity to ragweed Ag. Nevertheless, the exact numbers of CD8⁺CD60⁺CD45RO⁺ T cells that were ragweed specific, rather than T cells recruited into the ragweed IgE response, is unknown. It seems reasonable to infer that during peak ragweed allergy season, ragweed-specific memory CD8⁺CD60⁺ T cells divided in response to this allergen in lymphoid organs (MALT?) and emigrated and entered the circulation, whereas after ragweed allergy season, when allergen-induced cloning decreased, the numbers of ragweed-specific blood CD8⁺CD60⁺ T cells decreased. This idea is supported by the fact that high numbers of CD8⁺CD60⁺CD45RO⁺ T cells were present in blood of one extensively studied RS human at the peak of two ragweed allergy seasons (Fig. 4) and dramatically decreased thereafter to levels observed in NA humans (Fig. 1 and Table II). Nevertheless, because all RS humans in our study also were sensitized to other allergens, it would follow that CD8⁺CD60⁺ (CD45RO⁺) T memory cells related to these allergens might also be present in blood.

In the present studies, the highest numbers of CD8⁺CD60⁺ T cells were identified in blood during ragweed allergy season, after which their numbers dramatically decreased, but there were no significant decreases in total serum IgE levels. The lack of correlation between numbers of CD8⁺CD60⁺ T cells and serum IgE levels might be explained by the fact that all RS humans also were

CD8⁺CD60⁺ T cells (0.01–1.0 × 10⁵) were added back to PBMC (2 × 10⁶) depleted of these cells and PBMC cultured for 10 days with ragweed Ag, ragweed-specific IgE responses were restored (Fig. 6B). In contrast, when higher numbers of purified CD8⁺CD60⁺ T cells (10.0 × 10⁵) were added back to the depleted PBMC (2 × 10⁶), IgE responses were totally suppressed. Furthermore, when low numbers of purified CD8⁺CD60⁺ T cells (0.01–1.0 × 10⁵) were added back to unfractionated PBMC (2 × 10⁶) and PBMC cultured for 10 days with ragweed Ag, ragweed-specific IgE responses were induced (Fig. 6C). Again, when higher numbers of CD8⁺CD60⁺ T cells (10.0 × 10⁵) were added back to unfractionated PBMC (2 × 10⁶), IgE responses were totally suppressed.

CD4⁺ T cells. Previous studies by others determined that CD4⁺ T cells are required for in vitro induction of memory IgE responses (1, 2). In the present studies, no ragweed-specific IgE responses were induced when PBMC were depleted of CD4⁺ T cells and cultured for 0–12 days with ragweed Ag. These responses were completely restored when purified CD4⁺ T cells (10.0 × 10⁵) were added back to depleted PBMC (2 × 10⁶; data not shown).

Cytokine requirements for in vitro induction of ragweed-specific memory IgE responses

CD8⁺CD60⁺ blood T cells of RS humans produced IL-2, IL-4, IL-10, IL-12, IFN-α, and IFN-γ (23–40%), but not IL-6 or IL-13 (<1%; Fig. 7A); their CD4⁺ T cells also produced IL-4 (~30%; data not shown; other cytokines not studied).
skin test positive to other allergens. Their blood was obtained at the peak of the ragweed allergy season; therefore, many, if not most, of the CD8+CD60+ (CD45RO+CD60+) T cells were likely to be ragweed-specific memory cells, whereas serum IgE probably was directed against several allergens, including ragweed, so that no correlation would be expected because of the multiple specificities. Nevertheless, ragweed-specific IgE remains to be quantified in and out of ragweed allergy season, and it is possible that changes in CD8+ CD60+ T cell numbers will directly correlate with changes in ragweed-specific IgE.

The present studies have shown that a subset of CD8+CD60+ T cells (~20%) simultaneously expressed CD23, in contrast to <1% in serum IgE+ NA humans. Therefore, it is important to better define the identities of the CD8+CD60+ T cell subset (CD23+T) that help and/or suppress memory IgE responses in order that cells/cytokine pathways might be accurately determined. Because CD23 is shed from cell surfaces (11–13), it could be that in vivo, even more CD8+CD60+CD45RO+ T cells of RS subjects coexpressed CD23. There is substantial literature implicating CD23+ cells, T and B, and soluble CD23 as regulators of IgE responses (reviewed in Refs. 14–17). If so, CD23+ T cells that coexpress CD8 and CD60 may distinguish the e-specific helper or suppressor cells from CD8+ T cells that are known to help IgG (8, 9) and CD60+ T cells that are known to help IgG and IgA (10) responses and permit their selective deletion. However, the present experiments lay the groundwork for isolation and functional studies of this potentially important T cell subset.

The present studies confirm the well-known requirement for CD4+ T cells and IL-4 for induction of human IgE responses (reviewed in Refs 1 and 2). However, our studies also demonstrated that two distinct T cell subsets, CD4+ and CD8+ CD60+, are required for induction of memory IgE responses, extending the network(s) leading to memory IgE responses to include CD4+ (18, 19) and CD8+CD60+ T cells and cytokines produced by CD8+CD60+ T cells (IL-2, IL-4, IL-10, IL-12, IFN-α, IFN-γ; IL-4 also was produced by CD8+ T cells; other cytokines were not studied). Interestingly, in the present studies, IL-13 was not required for induction of memory IgE responses. The foregoing notwithstanding, it is possible that these cytokines are also produced by other PBMC.

Although the present studies are the first to demonstrate the strict requirement for two distinct T cell subsets, CD4+ and CD8+ CD60+ for induction of human memory IgE responses, earlier studies of Herrick et al. (3) were the first to demonstrate the requirement for two T cell subsets: Thy1+ asialo GM1 ganglioside-negative and Thy1+ asialo GM1 ganglioside-positive for induction of murine hapten-specific memory IgE responses. Furthermore, the observations with cytokines in the present human studies directly parallel those of Herrick et al. (3). Both studies demonstrated that IL-4 was responsible for ~50% of the in vitro memory IgE response and that IFN-γ and IFN-α also were required, with IFN-α either helping or suppressing these IgE responses, depending on its concentration. In the present studies, human memory IgE responses additionally required IL-2, IL-10, and IL-12, but not IL-13 (or IL-6).

In the present study, help (or potentiation) or suppression of memory IgE responses was observed when purified CD8+ CD60+ T cells, which made IL-2, IL-4, IL-10, IL-12, IFN-α, and IFN-γ, interacted in different proportions with PBMC of RS donors, which had been depleted of these cells, or with unfractionated PBMC. The mechanism(s) by which addition of low numbers of CD8+CD60+ T cells back to the depleted PBMC restored the ability of ragweed Ag to induce memory IgE responses, whereas addition of higher numbers totally suppressed these responses, are unknown. Because low numbers of CD8+CD60+ T cells and IFN-α helped memory IgE responses, whereas higher numbers and IFN-α suppressed them, an attractive possibility is that CD8+CD60+ (CD45RO+CD23+) IFN-α+ T cells secrete IFN-α, which directly or indirectly helps and/or suppresses memory IgE responses. This hypothesis is made more attractive by the fact that two T cell subsets and IFN-α performed similar helper and suppressor functions in mice (3).

The present findings with CD8+CD60+ T cells resemble earlier findings of Haskell and Axelrad (20) and Haskell and Harbrook (21), who showed that addition of low numbers of large spleen cells from mice primed with sheep erythrocytes suppressed plaque-forming cell responses of small lymphocytes, whereas higher numbers failed to do so. Konttainen and Feldmann (22) found carrier-specific suppression with low numbers of KLH-primed cells, as low as 1 × 10^5, in a system which tested helper activity as the target of suppression. Durkin et al. (23) demonstrated that low numbers of OVA-sensitized T memory cells added to higher numbers of lymph node cells from the same OVA-sensitized rats sometimes helped or potentiated and sometimes totally suppressed proliferative responses to either OVA or phytohemagglutinin. In the present studies, as was true of the earlier studies by others (20–23), the mechanisms involved in the network(s) leading to help/potentiation or suppression of memory responses obviously are complex, involving other cells and mediators released by cells participating in the memory response and feedback loops (24, 25).

The mechanism(s) by which CD8+CD60+ T cells helped IgE responses in the present studies is unknown, including the exact cell-cell and cell-cytokine interactions involved. Unraveling these mechanisms will require studies such as use of chamber experiments (26, 27). These studies would determine whether, for example, CD4+ T cells interacted with CD8+CD60+ T cells, or vice versa, or with intermediary cells to induce memory IgE responses. The requirement for six cytokines (IL-2, IL-4, IL-10, IL-12, IFN-α, and IFN-γ), each of which was produced by ~25% of CD8+CD60+ T cells, with IL-4 also produced by CD4+ T cells, and the probability that some these cytokines also might be produced by other PBMC, points to the fact that CD8+CD60+ T cells probably acted indirectly to induce memory IgE responses.

There is substantial literature related to CD8+ T cell-mediated killing (28–31). In the present studies, it remains to be determined whether suppression of memory IgE responses observed with higher numbers of CD8+ CD60+ T cells than those required for helper activity involved killing mechanisms. However, if CD8+CD60+ T cells prove to kill other PBMC to suppress memory IgE responses, this could be a mechanism by which IgE responses, in general, are selectively suppressed.

With respect to requirements for two distinct T cell populations for in vitro induction of specific memory IgE responses observed by Herrick et al. (3), Thy1+ asialo GM1 ganglioside negative and Thy1+ asialo GM1 ganglioside positive, and, in the present studies CD4+ and CD8+CD60+, it should be mentioned that CD60 also is a ganglioside (disialoganglioside II′(Neu5Ac)2-LacCer(9-O-acetyl Gc3) (10), albeit a different ganglioside from asialo GM1 ganglioside. This, of course, raises the question of whether or not it is simply happenstance that one of the two T cell subsets required for memory IgE responses in the murine and human systems coexpress gangliosides or if the gangliosides play an important role in generation of memory IgE responses, and this is the subject of our ongoing studies.

Lastly, it should be mentioned that in studies in our laboratory, when PBMC obtained from serum IgE+ RS donors outside the allergy season were cultured with ragweed Ag, IgE frequently was
not induced (>60% of experiments). When blood was obtained in season, IgE was not produced in ~25% of experiments. The reason(s) for this is unknown. One explanation for the successful induction of ragweed-specific memory IgE responses reported in the present studies is the fact that a requirement for induction of ragweed-specific memory IgE responses was met because blood was obtained in ragweed allergy season and contained the requisite numbers of CD8^+CD69^+ T cells required for induction of these responses. This, however, does not rule out that there may be additional unknown requirements for induction of memory IgE responses, which have been met in the present studies by obtaining PBMC during peak ragweed allergy season.

Disclosures
The authors have no financial conflict of interest.

References

Downloaded from www.jimmunol.org by guest on April 28, 2017