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Human Interdigitating Dendritic Cells Induce Isotype Switching and IL-13-Dependent IgM Production in CD40-Activated Naive B Cells

Bengt Johansson,* Sigurdur Ingvarsson,* Pia Björck,† and Carl A. K. Borrebaeck2*

Interdigitating dendritic cells (IDC) represent a mature progeny of dendritic cells (DC) in vivo and are exhibiting a strong lymphocyte stimulatory potential. Because of the restricted localization to secondary lymphoid organs where decisive cellular interactions take place in the initial events of immunity, IDC regulatory function was addressed in relation to naive B cells. In this study, we demonstrate that human tonsillar IDC induce a dual response from CD40-activated IgD+/CD38− naive B lymphocytes. IDC direct naive B cells toward either isotype switching or an IL-13-dependent IgM secretion. IDC-dependent proliferation, isotype switching, and Ig production are all strictly mediated by soluble factors, suggesting that such skewing in B cell activation is the result of differential cytokine expression. Moreover, IDC-expressed IL-13 represents a novel source of a cytokine with recently established effects in Th2 induction as well as in immunological disorders resulting in allergic reactions. The Journal of Immunology, 2000, 164: 1847–1854.

Materials and Methods

Reagents and Abs

FITC-conjugated anti-CD3, anti-CD14, and anti-CD20 as well as PE-conjugated anti-CD2, anti-CD38, anti-HLA-DR, and anti-IL-13 (JES10–5A2) mAbs were purchased from Becton Dickinson (San Jose, CA). FITC-conjugated anti-CD1a, anti-CD11c, anti-CD19, and swine anti-rabbit Ig, and PE-conjugated anti-CD4 and anti-CD25 mAbs were obtained from Dako (Glostrup, Denmark) as was a primary mAb against human HLA-DR, DQ, DR (mouse γ1), biotinylated F(ab′)2 fragment of rabbit anti-mouse Ig and alkaline phosphatase-conjugated streptavidin. FITC-conjugated anti-CD80 and anti-CD83 mAbs were purchased from Immunotech S.A. (Marseille, France). Anti-CD86 mAb (Bu63), a generous gift from John Pound (Birmingham, U.K.), was purified from ascites fluid by ion exchange chromatography on SP-Sepharose (Pharmacia Biotech, Uppsala, Sweden) and conjugated to FITC according to standard protocols. Anti-CD40 (S2C6) mAb was a generous gift from Staffan Pauli (Stockholm University) and was biotinylated according to standard procedures. FITC-conjugated

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0222-1767/00/$02.00
F(ab')2 fragment of goat anti-human IgD was obtained from Caltag (Burlingame, CA) as was tri-color-conjugated streptavidin used for detection of biotinylated CD40 mAb. Primary anti-REiB Ab (rabbit) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA).

**Purification of IDC from tonsils**

Human tonsils were obtained from children undergoing tonsillectomy at Lund University Hospital (Lund, Sweden) or at Malmö Academic Hospital (Malmö, Sweden). IDC were purified as described previously (3). Briefly, tonsils were cut into fragments in RPMI 1640 (Life Technologies, Paisley, Scotland) supplemented with 50 μg/mL gentamicin (Biological Industries, Hamek, Israel). Tonsil fragments were incubated with or without 10 mU/mL DNase I (Sigma, St. Louis, MO) and 50 μM Deoxyribonuclease I (Sigma). For each round, tissue fragments were incubated with both enzymes for 20 min at 37°C. Cells were washed once in gentamicin-supplemented RPMI 1640, layered on 50% isonicotic Percoll (Pharmacia Biotech), and centrifuged at 900 × g for 20 min. Cells in the interphase were washed in RPMI 1640 with gentamicin, and CD19+ B cells were depleted by two rounds of selection using anti-CD19-coated magnetic beads (Dynal, Oslo, Norway). Negatively selected cells were incubated with a biotinylated mAb against CD40 (25C6) and FITC-conjugated mAbs against CD3, CD14, CD20, and CD19. CD40 labeling was visualized by tri-color-conjugated streptavidin. CD40εβ and FITC-negative IDC were sorted using a FACS Vantage (Becton Dickinson). Cell sorting was performed at a speed not exceeding 2500 events/s. Sorted cells were routinely >97% pure, as determined by FACSscan (Becton Dickinson).

**Isolation of naive B cells**

Autologous IgD+/CD38− naive B cells were obtained from tonsillar cell suspensions, removed before the treatment with digestive enzymes. High-density cells were enriched in the pellet of a 50% Percoll gradient and washed twice in gentamicin-supplemented RPMI 1640. Ethyrocyes were removed by centrifugation on Ficoll-Paque (Pharmacia Biotech). After being washed twice in RPMI 1640 with gentamicin, the mononuclear cell fraction was incubated with an FITC-conjugated Fab(′)2 fragment of goat anti-human IgD and a PE-conjugated mAb against CD38. IgD+/CD38− naive B cells were sorted using an FACS Vantage (Becton Dickinson). Sorted B cells had routinely a purity >98%.

**Flow cytometry**

All flow cytometric analyses were performed on a FACScan (Becton Dickinson). PBS containing 1% BSA (w/v) and 5 mM EDTA was used in all cell labeling and washing steps. Abs were titrated to optimal working dilutions on freshly isolated mononuclear cells from tonsils or peripheral blood, and appropriate isotype-matched controls were used to set background staining. Gates were set to exclude debris and nonviable cells on basis of light scatter properties. Freshly sorted IDC and IDC cultured overnight in complete medium (i.e., RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 1% (v/v) nonessential amino acids (Life Technologies, Rockville, MD), and 50 μg/mL gentamicin) were incubated with FITC-conjugated Abs to CD1a, CD4, CD11c, CD80, CD83, and CD86 and PE-conjugated Abs to CD2, HLA-DR, and CD25. Cells were also fixed and permeabilized with Ortho PermeaFix (Ortho Pharmaceuticals, Raritan, NJ) and stained with a primary Ab for intracellular expression of REiB using FITC-conjugated swine anti-rabbit Ig Ab as a secondary reagent.

For intracellular staining of IL-13, IDC were cultured for 10 h in complete medium with or without 5 ng/mL PMA and 5 μg/mL ionomycin (Sigma) and for 2 additional h with 5 μg/mL brefeldin A. Recovered cells were first stained for surface expression of CD83 (FITC-conjugated mAb) and subsequently for intracellular IL-13 (PE-conjugated mAb) using Ortho PermeaFix according to the manufacturer’s guidelines.

**Immunostaining of IDC**

To visualize morphological features of IDC, cytopsins were prepared either directly after sorting or after a 12-h culture in complete RPMI 1640 medium. Cells were centrifuged (3 min at 300 rpm) onto microscope slides and fixed in ice-cold acetone for 5 min. After rinsing with PBS, cells were stained with a primary mAb (mouse anti-human HLA-DR, DQ, DR), secondary reagent (biotinylated F(ab')2 fragment of rabbit anti-mouse Ig) followed by alkaline phosphatase-conjugated streptavidin (Dako A/S). Slides were developed with a Dako Fast Red substrate system (Dako, Carpinteria, CA). PBS was used for all dilutions of regents and all washing steps.

**Cocultures of naive human B cells and IDC**

A total of 10^4 IgD+/CD38− B cells were cultured in flat-bottom 96-well tissue culture plates (Costar, Cambridge, MA) in the presence or absence of 10^4 IDC in a final volume of 200 μL of complete RPMI 1640 medium. IDC as well as naïve B cells were cocultured with γ-irradiated (70 Gy) CD32/CD40 ligand (CD40L)-transduced mouse fibroblasts (2.5 × 10^3 cells/well) to provide efficient CD40 ligation. Nontransfected cells were used as a control. To analyze the effects of IDC-produced IL-13 on B cells, in other experiments a neutralizing mAb against human IL-13 (mouse y1, clone 32116.11; R&D Systems, Minneapolis, MN) or an isotype-matched irrelevant mAb (R&D Systems) was added to IDC cultures, both at 50 μg/mL; the latter mAb had no effects on any of the analyzed parameters. Flow cytometry was performed after 6 days by [methyl-3H]thymidine incorporation (0.5 μCi/well; Amersham, Arlington Heights, IL) using an 18-h pulse period. Igs were measured in culture supernatants after 14 days, and detection of IgM, IgG, and IgA was performed using ELISA with isotype-specific Abs (Zymed, South San Francisco, CA), as described (27). All cultures were conducted in triplicate and are represented as the mean values ± SD.

**Transwell cell culture experiments**

Requirements for membrane-bound vs soluble signals in IDC-induced proliferation and isotype switching of CD40-activated naïve B cells were analyzed by separating B cells and IDC using transwell inserts (Costar). In 24-well tissue culture plates (Costar), 1.5 × 10^4 naïve B cells were cultured in the lower compartment along with 5 × 10^4 irradiated CD32/CD40L-transfected fibroblasts. The upper compartment contained 1.5 × 10^5 IDC, CD40-triggered by 5 × 10^3 CD32/CD40L-transfected fibroblasts. For comparison, transwell inserts were omitted and B cells were cocultured with the double transfectants either alone or in combination with IDC. Independent of culture system used, cells were cultured in a final volume of 50 μL of complete RPMI 1640 medium. Supernatants were analyzed for Ig production after 14 days as described above. The proliferative response of B cells in the lower compartment was monitored after 6 days by [methyl-3H]thymidine incorporation (2.5 μCi/well; Amersham) incorporation. After an 18-h pulse period, cells were recovered with 5 mM EDTA in PBS, centrifuged, resuspended in 200 μL PBS and analyzed in a scintillation counter.

**RNA extraction and cDNA synthesis**

A total of 10^3 sorted IDC cultured with 2.5 × 10^5 irradiated CD32/CD40L-transfected fibroblasts for 24 h were recovered, washed twice in PBS, and lysed in 200 μL of 4 M guanidine thiocyanate in 10 mM Tris-HCl buffer (pH 7.5). Polyadenylated RNA was extracted using magnetic capture on oligo(dT),25 beads (Dynal) as described (28). Polyadenylated RNA, attached to beads, was reversely transcribed using Moloney murine leukemia virus-RT (200 U; Life Technologies) for 1 h at 37°C in 20 μL containing RNasin (1 U; Promega, Madison, WI), 10 mM DTT (Life Technologies), 1 mM dNTP mixture (Pharmacia, Piscataway, NJ), 100 μg/mL BSA (IBU; Kodak, New haven, CT) and 1 × Moloney murine leukemia virus-RT buffer in diethyl/pyrocatechol-treated water.

**RT-PCR analysis of cytokine and CD marker transcripts**

A total of 1 μL of the cDNA reaction described above or 1 μL of cDNA obtained in the same manner from PBMC activated for 48 h with PMA (5 ng/mL) and ionomycin (5 μg/mL) was amplified in a PCR. Primers were designed to amplify cDNA fragments that could clearly be distinguished from corresponding genes by deleted introns (do not apply to CD19 since this protein is encoded by only one uninterrupted exon). PCRs were performed in 50 μL containing AmpliTaq DNA polymerase (2.5 U; Perkin-Elmer/Cetus, Norwalk, CT), 800 nM of the 5′ and 3′ primers (Table I), and 125 μM dNTPs in 1 × PCR buffer (Perkin-Elmer/Cetus). Reactions were performed in a Perkin-Elmer GeneAmp PCR system 2400 with 40 cycles (94°C for 1 min, 55°C for 1 min, and 72°C for 2 min). Ten microliters of PCR products was analyzed on a 2% agarose gel in Tris-acetate-EDTA buffer containing 0.25 μg/mL ethidium bromide. PCR with cDNA template from CD32/CD40L-transfected mouse fibroblasts served as a negative control for each primer pair and did not result in amplified products.

**IL-13 ELISA**

Sorted IDC were cocultured with CD32/CD40L transfectants with or without the presence of B cells, using a cell ratio of 4:1:4, in complete RPMI 1640 medium. Depending on the yield of IDC in each individual experiment, IDC were seeded at a cell density of 4 × 10^4 cells/mL. Control cultures with only B cells and CD32/CD40L transfectants were also performed. Cell-free supernatants were recovered after 3 days and IL-13 concentrations were analyzed using a Quantikine ELISA kit for human IL-13 (R&D Systems). Production is expressed as pg of IL-13/10^5 IDC.
Results

Interdigitating cells were isolated based on in situ observations of a CD40<sup>high</sup> expression and lineage-negative phenotype (3, 29). After enrichment of low-density leukocytes, remaining cells were depleted of CD19<sup>+</sup> cells by magnetic beads, and cell sorting of CD40<sup>+</sup>CD3<sup>-</sup>CD14<sup>-</sup>CD19<sup>-</sup>CD20<sup>-</sup> cells by flow cytometry (>97% purity) was performed. The isolated cells displayed a typical dendritic phenotype (Fig. 1, A and C) and morphology as visualized by MHC class II staining of prepared cytospins (Fig. 1B). Sorted cells stained strongly for MHC class II, but only

---

Table I. Oligonucleotide primers used for amplification of cytokine and CD marker cDNA with sizes for PCR products corresponding to amplified mRNA

<table>
<thead>
<tr>
<th>mRNA</th>
<th>Primer Sequences (Forward/Reverse)</th>
<th>PCR Product Size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Actin</td>
<td>5′-GTGGGGCCGCCACCCAGCACC-3′&lt;br&gt;5′-CTCCTTAATGTCACGCCAGATTTC-3′</td>
<td>540</td>
</tr>
<tr>
<td>IL-6</td>
<td>5′-CAGTTGCTTCTCTCCTTTGGGCTCCT-3′&lt;br&gt;5′-TGTTCAATGTCCGCCACCACGTTC-3′</td>
<td>540</td>
</tr>
<tr>
<td>IL-10</td>
<td>5′-AGATCTCCGAGATGCGCTCA-3′&lt;br&gt;5′-TITCGTATCTTCATTCTGATGA-3′</td>
<td>408</td>
</tr>
<tr>
<td>IL-13</td>
<td>5′-GCCCTTCTTTGTGACCCAGTGTCATT-3′&lt;br&gt;5′-TGTGGTGTCTCCGACATGCAAG-3′</td>
<td>320</td>
</tr>
<tr>
<td>CD14</td>
<td>5′-GAAGACTTATCAGCCTTAGAG-3′&lt;br&gt;5′-GGCAAGCTGCTAGTACCTGGA-3′</td>
<td>550</td>
</tr>
<tr>
<td>CD19</td>
<td>5′-AGCTGTTCGCGGGAATGTGTT-3′&lt;br&gt;5′-CTAGGCTCAGCAATGACTTAG-3′</td>
<td>327</td>
</tr>
<tr>
<td>TCR-α</td>
<td>5′-CACAAAGTAGTTAAGGTTCTGAGTTC-3′&lt;br&gt;5′-ATCATAAATTCTCAGGAGATCC-3′</td>
<td>494</td>
</tr>
<tr>
<td>CD83</td>
<td>5′-TCGCGGCGCCACCTCCGACTCTGCT-3′&lt;br&gt;5′-TACAGTTTCTGTCTGTGAGG-3′</td>
<td>612</td>
</tr>
</tbody>
</table>

FIGURE 1. Sorted IDC display morphology and phenotype characteristics of lineage-restricted DC and culturing overnight in complete RPMI 1640 medium only further develops these characteristics to correspond to fully matured DC. A, sorting of IDC based on a lineage-negative phenotype (lack of CD3, CD14, CD19, and CD20) and high CD40 expression. Sorted IDC routinely had a purity of >97%. B, Staining of cytospin prepared with IDC directly after sorting (day 0) with a mAb to MHC class II reveals dendritic protrusions, which are clearly elongated after culturing overnight (day 1). C, FACS profiles of proteins expressed by IDC directly after sorting (day 0) or after overnight culture (day 1). Cells were stained with FITC- and PE-conjugated mAbs to indicated proteins (black histograms) or with conjugated isotype-matched controls (gray histograms).
weakly for CD1a. Both CD4 and CD11c were expressed on isolated cells, although CD4 expression was significantly reduced or completely lost after 14 h in culture. Levels of CD80 and CD86 were generally low on freshly purified IDC but these markers were strongly up-regulated after culture, as was the IL-2 receptor (CD25). Furthermore, the transcription factor RelB, a member of the NF-κB protein family shown to be involved in differentiation and maturation of DC (30), was abundant in all IDC as assessed by intracellular staining. The DC-associated maturation marker CD83 is present on a subpopulation of IDC within the paracortex of tonsils and the periarteriolar lymphoid sheaths of spleen (3). Directly after isolation, the majority of the IDC were negative for CD83, but expression of this marker was up-regulated after in vitro culture to a level that correlated well with in situ observations. Moreover, activation of protein kinase C and calcium-dependent signaling pathways by PMA and ionomycin generated a homogenous CD83 expression on almost 100% of the cells (data not shown). Finally, neither CD3- nor CD19-positive cells could be detected in cultures, eliminating the possibility of contaminating T and B cells in the isolated IDC population. Thus, the morphology and phenotypic characteristics confirm that the isolated cells indeed represent lineage-restricted DC, which after a short period in culture develop the phenotype of mature IDC.

**IDC directly induce isotype switching in CD40-activated naive B cells**

IDC were sorted on their CD40 expression and consequently received some stimulation from this procedure, whereas CD40 activation of naive B lymphocytes was accomplished by presenting the CD40L on transfected mouse fibroblasts. However, to achieve the most efficient CD40-dependent activation of both B cells and IDC, sorted cells were seeded onto irradiated CD32/CD40L double transfected mouse fibroblasts. When subsequently analyzing the cultures for soluble Ig production, the IDC-dependent response was heterogeneous between different donors. Fig. 2A shows this response from 17 independent experiments represented as the relative amount of switched Ig produced in the presence of IDC as a function of the IDC-dependent increase in total secretory Ig. The presence of IDC resulted in, for the great majority of donors, a substantial increase in soluble Ig (≥2-fold increase in 15/17 experiments). IDC from some donors enhanced the soluble IgM production but had little effect on the production of downstream isotypes (Fig. 2B), whereas IDC from other donors triggered CD40-activated naive B cells to undergo significant isotype switching and produce IgG and IgA (Fig. 2C). A high ratio of isotype-switched Ig seems to correlate to a relatively low increase in total Ig and vice versa. The control, where only B cells were seeded onto CD32/CD40L-transfected fibroblasts, produced very low levels of IgM and secretion of IgG and IgA was not detectable. IDC, not only increasing the production of soluble Ig, but also capable of dramatically enhancing the ratio of IgG and IgA to IgM, consequently can induce isotype switching in CD40-activated naive B cells.

**IDC-induced isotype switching in CD40-activated naive B cells is mediated by soluble factors**

The proliferation of CD40-activated naive B cells induced by in vitro-generated DC is dependent on, so far, unidentified soluble signals, whereas plasma cell differentiation has been shown to involve DC-produced IL-12 (31). To evaluate the requirement for physical interactions between different cell types during IDC-induced isotype switching, sorted IDC and naive B cells were compartmentalized by a permeable membrane. Both compartments contained CD32/CD40L transfectants to provide CD40 stimula-

![FIGURE 2](http://www.jimmunol.org/)
Cytokine production of CD40-activated IDC

Since IDC are capable of enhancing CD40-mediated proliferation, isotype switching to IgG/IgA, as well as Ig secretion through soluble factors, the presence of transcripts for IL-6, IL-10, and IL-13 were assessed by RT-PCR. All of these cytokines have well-documented effects in Th2-mediated responses involving switch and Ig secretion (24, 25, 32, 33). IL-4, a key cytokine in Th2-type reactions (34), has not been detected in culture supernatants from CD40-ligated IDC (3) nor could specific mRNA be amplified from in vitro-generated DC (35) or blood DC (36) with RT-PCR. We demonstrate here the presence of IL-13 transcripts in CD40-activated IDC using specific primers to amplify cDNA of the expected size (Fig. 4). Gene transcription of this particular cytokine varied between different donors since amplification was only achieved in two of four donors, whereas control amplification of β-actin was used to standardize total mRNA content in performed PCRs.

FIGURE 3. IDC-dependent isotype switching, Ig secretion, and proliferation of CD40-activated naive B lymphocytes are exclusively mediated by soluble factors. A, Highly purified naive B cells (150,000) were cultured in the absence or presence of sorted IDC (150,000), either in the same compartment or separated by a transwell insert, as described in Materials and Methods. CD40 ligation of cells in each compartment was accomplished by CD32/CD40L double transfected fibroblasts. Culture supernatants were harvested after 14 days and IgM, IgG, and IgA were measured. B, Requirements for physical interaction in IDC-induced proliferation of CD40-activated B cells were assessed by separating cells with transwell inserts. [methyl-3H]Thymidine incorporation into DNA was analyzed for naive B cells (150,000) cultured for 6 days (18-h pulse period) stimulated by nontransfected fibroblasts (none), sorted IDC (150,000), or CD32/CD40L-transfected fibroblasts, or with transfected fibroblasts and sorted IDC, where IDC were seeded either in the same compartment or in a transwell insert along with CD32/CD40L-transfected fibroblasts.

FIGURE 4. CD40-activated IDC express mRNA for IL-13 as well as for IL-6 and IL-10. RT-PCR analysis of transcripts for cytokine and CD markers present in CD40-activated IDC. PMA/ionomycin-activated PBMC served as a positive control (+) for each primer pair used (Table I) and yielded PCR products of expected mRNA size. mRNA from CD32/CD40L-transfected mouse fibroblasts were used as a negative control and did not result in any detectable amplification. mRNA levels of β-actin were used to standardize total mRNA content in performed PCRs.

Cytokine production of CD40-activated IDC

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Next, we investigated IL-13 expression at the protein level by intracellular staining of IDC cultured for 10 h in complete medium using flow cytometry. To avoid contaminating CD32/CD40L-transfected fibroblasts in the collected data, these cells were omitted in cultures. CD40 activation could still be accomplished by the remaining cell-bound anti-CD40 mAb from the positive selection of IDC. As can be seen in Fig. 5, IDC clearly expressed intracellular IL-13. The requirement of CD40 stimulation for these IDC to produce IL-13 could not be evaluated, since some degree of activation via CD40 most certainly is obtained as a consequence of the cell-sorting procedure. The production of IL-13 was, however, not dependent on the exogenous addition of PMA and ionomycin. Addition of these reagents to cultures did not significantly alter IL-13 staining of analyzed cells (data not shown). However, the variation in mRNA between different donors was also confirmed at the protein level as the number of IL-13-positive cells varied from <5% of the cells to >80% (n = 4) among different donors.
Anti-IL-13 inhibits the IDC-induced Ig production

The action of IDC-derived IL-13 on Ig production was analyzed by the addition of a neutralizing anti-IL-13 mAb to the cultures. At a concentration of 50 μg/ml, the IL-13-specific mAb inhibited IDC-induced IgM production by 54% (range, 15–86%; n = 4) (Fig. 6A; Table II). Similar reduction was also seen for IgG in an experiment where IDC induced a significant secretion of this isotype (Expt. 4, Table II). In all IL-13-neutralizing experiments, IgA responses were too low for an accurate measurement of inhibition. Furthermore, the reduced Ig production was not merely a result of decreased proliferation, since [3H]thymidine incorporation was not effected (Fig. 6B). Although the maximum observed inhibitory effect of 86% argues for an essential role of IL-13 in the IDC-driven IgM production, the lower degree of inhibition observed in other experiments suggests that other IDC-derived factors also may be involved in this process. To analyze donor-dependent differences of IL-13, the concentration in culture supernatants of IDC stimulated for 3 days on CD32/CD40L-transfected fibroblasts was measured by ELISA. IDC isolated from three different donors produced 30, 110, and 260 pg/106 cells, respectively, and these values changed <5% if B cells also were included in cultures. IL-13 was not detected in supernatants from CD40-stimulated B cells only. In summary, these data demonstrate that IL-13 produced by IDC drives CD40-stimulated naive B cells to produce IgM. Also, secretion of IgG might involve IL-13 (Table II), but IDC-dependent isotype switching, exclusively mediated by soluble factors as shown herein, still needs further research for a complete understanding of its mechanism.

Table II. Inhibition of IDC-dependent IgM and IgG production from CD40-activated naive B cells in the presence of a neutralizing anti-IL-13 mAb (50 μg/ml)

<table>
<thead>
<tr>
<th>Expt.</th>
<th>IDC-Dependent Ig Secretion (ng/ml)</th>
<th>Inhibition by Anti-IL-13 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>1</td>
<td>231</td>
<td>—</td>
</tr>
<tr>
<td>2</td>
<td>260</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>152</td>
<td>—</td>
</tr>
<tr>
<td>4</td>
<td>46</td>
<td>75</td>
</tr>
<tr>
<td>mean value</td>
<td>54</td>
<td>43</td>
</tr>
</tbody>
</table>

*In all experiments, the IgA response elicited by IDC was too low (<10 ng/ml) for adequate measurement of inhibition as was the IgG response where indicated (—). Experimental conditions are described in Fig. 6.

Discussion

Recent studies have demonstrated that DC are a highly versatile group of APC capable of directly stimulating B cells, including CD40-activated IgD+ naive B cells, to become IgM-secreting plasma cells (3, 11, 23, 31). In this study, we show that IDC, when isolated and studied ex vivo, can induce proliferation and differentiation toward IgM-secreting cells and are also capable of enhancing CD40-mediated isotype switching in naive B cells leading to both IgG and IgA secretion.

DC-dependent induction of a humoral immune response has been described previously (37–39). In addition, in mice with an insertional mutation disrupting the gene coding for RelB, manifested by the lack of functional DC, impairment in switching and alterations in isotype composition were observed when animals were challenged by defined Ags (30, 40). In all of these reports, the effects of DC on isotype switching have been mediated via interactions between DC and T cells, or the contributions of T cells have not been possible to estimate or to completely exclude. Although isotype switching requires the involvement of the CD40 molecule and its ligand provided by T cells (41), the direct involvement of DC in this process was recently shown. DC derived...
from CD34+ HPC induced expression of surface IgA on CD40-activated naive B cells (42). This direct effect on B cells did not include the up-regulation of surface IgG, and neither soluble IgA nor IgG could be detected without exogenous addition of IL-10 and TGF-β. On the other hand, our results provide evidence for IDC-induced switching, including IgG, in CD40-activated naive B cells with subsequent secretion of IgG and IgA, generally with IgG being the most dominant switch product. PCR-based analysis to further dissect which different isotype subgroups being induced by IDC switching is presently undertaken. So far, productive transcripts for IgG2, IgG3, and IgA2 have been specifically amplified with cDNA derived from IDC/B cell cultures (our unpublished observations). Beside the absence of these mRNA species in B cells only activated through the CD40 molecule, transcripts were also undetectable in sorted cell populations after CD19/GAPDH standardization of total cDNA.

The reciprocal relationship of class switching and Ig secretion observed in IDC-dependent activation of B cells suggests that IDC have the potential to regulate an immune response to either plasma cell differentiation and high IgM production or to a differentiation pathway resulting in isotype switching. IL-6 and IL-10 have in combination with IL-4 been shown to possess a capacity to modulate differentiation of CD40-activated naive B cells, where IL-10 promotes switching in parallel to phenotypic changes resulting in centoblastic features (24, 25). Thus, the expression of cytokines from IDC, including IL-6, IL-10, and IL-13, could indicate that these cells have a more complex regulatory function in humoral vs cellular immunity, coordinating B and T cells by a skewed production of cytokines. The occurrence of different subsets of DC, inducing production of type 1 and type 2 cytokines from T cells, has also recently been reported (43, 44).

Data supporting the effect of IL-13 on naive B cells has, during the course of this study, been reported by others. When analyzing expression of the αα subunit of the IL-13R (IL-13Rα1) on human tonsillar B cells, highest levels were found on IgD+/CD38+ cells and expression was further up-regulated by CD40L but absent on CD38- cells (45). These observations suggest a high susceptibility for IL-13 by IgD+/CD38- naive B cells, especially when activated with CD40L. In accordance with this, IgD+/CD38- B cells and IDC colocalize in the T cell area of tonsil sections (3), further supporting an in vivo relevance for an interaction among B cells, IDC, and T cells. In another report, mouse IL-13 was shown to enhance IgM production from CD40-activated IgD+ naïve murine B cells (46). No evidence was found for a direct effect of IL-13 on isotype switching and proliferation. Thus, the effects by IL-13 on murine B cells were similar to the responses obtained in this study using human naive B cells and IDC producing IL-13.

It is worth noticing that plasma cell differentiation and IgM production induced by CD34+derived DC was critically dependent on IL-12 produced by these cells (31). As shown here, CD40-ligated IDC also readily produce IL-13, whereas in vitro-generated DC or the recently described germinal center DC (16) produced IL-13 only after stimulation with PMA and ionomycin (35). The difference in cytokine profile may be due to IDC and in vitro-generated DC 1) representing different stages of maturation, or 2) being of different origin with possibly different function in vivo. CD34-derived DC are likely to be of myeloid origin (10), whereas IDC with low or no expression of myeloid markers may be of lymphoid origin (3). Thus, although both in vitro-generated DC and IDC are able to promote B cell differentiation, the differences mentioned above may account for the discrepancies found between the two DC types both with regard to the levels of Ig produced as well as the induction of different Ig isotypes.

In a recent study (44), two types of vitro-generated DC were described, DC-1 of myeloid origin, capable of producing IL-12 and inducing a Th1 response, and DC-2 of lymphoid origin, giving rise to Th2 cells by a so far unknown mechanism. In this context, it would be interesting to examine whether IDC producing IL-13 can affect the Th2 development, if not by direct effect(s) on the T cells, since those lack functional IL-13 receptors, by indirect effect, i.e., macrophage-derived chemokines (47). The importance of IL-13 in Th2 development is also clear from studies using IL-13 knockout mice (48). Moreover, IL-13 is clearly being a key cytokine in allergic asthma (49, 50) and parasitic diseases such as Schistosoma mansoni (51) and nematode infections (52). Consequently, IL-13-producing IDC may play an important role in several of these diseases and clearly merit further study.

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References
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