Migration and Maturation of Human Colonic Dendritic Cells

Sally J. Bell, Rachael Rigby, Nicholas English, Steven D. Mann, Stella C. Knight, Michael A. Kamm and Andrew J. Stagg

*J Immunol* 2001; 166:4958-4967;
doi: 10.4049/jimmunol.166.8.4958
http://www.jimmunol.org/content/166/8/4958

**References**

This article cites 45 articles, 21 of which you can access for free at:
http://www.jimmunol.org/content/166/8/4958.full#ref-list-1

**Why The JI?** Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

**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
Migration and Maturation of Human Colonic Dendritic Cells

Sally J. Bell,*† Rachael Rigby,* Nicholas English,* Steven D. Mann,*† Stella C. Knight,* Michael A. Kamm,† and Andrew J. Stagg†*

Dendritic cells (DC) in the intestinal tract are likely to play a pivotal role in the initiation and regulation of immune responses. Studies in rodents suggest that small numbers of DC continually circulate through gut tissue with a transit time of a few days, and additional numbers are rapidly recruited in response to inflammatory stimuli (1). DC are present in the gut-associated lymphoid tissue, such as Peyer’s patches, and are scattered throughout the lamina propria, and a DC population might also be located within the epithelium itself (2–4). DC isolated from murine Peyer’s patches produce more IL-10 than their counterparts in the spleen and have a greater propensity to stimulate Th2 responses (5). Thus, the tendency of many Ags to induce Th2/3 responses at mucosal surfaces may result from the type of cytokine produced by DC at these sites.

DC may be involved in both responsiveness and nonresponsiveness to Ags in the gut. Gut DC can acquire Ags such as OVA when these are fed to an animal, and upon isolation these DC can activate OVA-specific T cells (6). In contrast, treatment of mice with the growth factor Flt3L, which boosts intestinal DC numbers, en-

Alterations in intestinal DC function could contribute to the poorly understood dysregulated immune responses that underlie the human inflammatory bowel diseases (IBD), Crohn’s disease (CD), and ulcerative colitis. However, little is known about DC in the human intestine. Cell populations with phenotypic properties consistent with DC have been identified in a number of immunocytochemical studies (12–14), and partially enriched populations of these cells have been shown to stimulate primary T cell responses in a primary allogeneic MLR (15). Detailed analysis of the phenotype and function of these cells is currently lacking.

In most nonlymphoid tissues DC are present as immature cells (reviewed in Ref. 16). These immature DC express low levels of co-stimulatory molecules and take up Ag very efficiently, but are poorly stimulatory for T cells. In response to maturation signals, which include microbial products and cytokines such as TNF-α, these cells change their pattern of expressed chemokine receptors and migrate to the draining lymphoid tissue. During this process, DC down-regulate their Ag acquisition machinery, up-regulate the cell surface expression of MHC-peptide Ag complexes and co-stimulatory molecules, and acquire their characteristic ability to

Copyright © 2001 by The American Association of Immunologists

0222-1767/01/$02.00

Received for publication September 6, 2000. Accepted for publication February 6, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

† Address correspondence and reprint requests to Dr. Andrew Stagg, Antigen Presentation Research Group, Imperial College at Northwick Park Hospital, Watford Road, Harrow, Middlesex, United Kingdom HA1 3UJ. E-mail address: a.stagg@ic.ac.uk

* Abbreviations used in this paper: DC, dendritic cell; CD, Crohn’s disease; IBD, inflammatory bowel disease; LPMC, lamina propria mononuclear cell; MFI, mean fluorescence intensity; UC, ulcerative colitis; d0, day 0; d1, day 1.

© 2001 by the American Association of Immunologists

0222-1767/01/$02.00

Migration and Maturation of Human Colonic Dendritic Cells

Sally J. Bell,*† Rachael Rigby,* Nicholas English,* Steven D. Mann,*† Stella C. Knight,* Michael A. Kamm,† and Andrew J. Stagg†*

Dendritic cells (DC) in the colon may regulate intestinal immunity but remain poorly characterized. In this study a CD11c⁺HLA-DR⁺lin⁻ (CD3⁻CD14⁻CD16⁻CD19⁻CD34⁻) population has been identified by flow cytometry in cells obtained by rapid collagenase digestion of human colonic and rectal biopsies. These day 0 (d0) CD11c⁺HLA-DR⁺lin⁻ cells comprised ~0.6% of the mononuclear cells obtained from the lamina propria, were endocytically active, and had the phenotype of immature DC; they were CD40⁺ and expressed low levels of CD38 and CD86, but little or no CD80 or CD25. Similar d0 DC populations were isolated from the colonic mucosa of healthy controls and from both inflamed and noninflamed tissue from patients with Crohn’s disease. The lamina propria also contained a population of cells capable of migrating out of biopsies during an overnight culture and differentiating into mature DC with lower levels of endocytic activity and high cell surface expression of CD40, CD80, CD86, CD38, and CD25. This mature DC population was a potent stimulator of an allogeneic mixed leukocyte (MLR). Overnight culture of cells isolated by enzymatic digestion on d0 yielded DC with a phenotype intermediate between that of the d0 cells and that of the cells migrating out overnight. Overnight culture of colonic cells in which DC and HLA-DR⁺lin⁺ cells were differentially labeled with FITC-dextran suggested that some of the maturing DC might differentiate from HLA-DR⁺lin⁻ progenitors. This study presents the first analysis of the phenotype, maturational status, and migratory activity of human gut DC. The Journal of Immunology, 2001, 166: 4958–4967.
stimulate naive T cells. It has recently been suggested that an abnormal pattern of DC maturation, such that mature cells fail to migrate but instead remain localized in the tissue, could underlie chronic inflammatory processes (17).

In the current study, we have used multicolor flow cytometry to characterize DC present in small amounts of biopsy tissue obtained from the human colon and for the first time have analyzed the phenotype and maturation state of DC present in tissue from inflammatory bowel disease patients and healthy controls.

Materials and Methods

Patients

Patients with active CD (n = 24), patients with ulcerative colitis (UC) undergoing surveillance (n = 14), and those undergoing colonoscopy for other reasons (e.g., polyp follow-up, family history of cancer, or investigation of rectal bleeding) who were subsequently found to have normal histology (controls; n = 22) were prospectively enrolled in the study (Table I). Six random colon biopsies were obtained from all UC and control patients and from 10 of the patients with CD. Three rectal biopsies were studied from 14 CD patients who were being assessed for anti-TNF-α treatment. The characteristics of the patients are described in Table I. Disease activity was assessed endoscopically with histological confirmation.

Processing of biopsies

Biopsies obtained during colonoscopy or sigmoidoscopy were collected in ice-cold Dutch modification of RPMI 1640 (Sigma, St. Louis, MO) supplemented with 10% FCS, 2 mM l-glutamine, gentamicin (25 μg/ml), and penicillin/streptomycin (100 U/ml) (complete medium). They were incubated on a shaker at 37°C, they were washed in HBSS until the supernatant was macroscopically free of released epithelial cells. The EDTA incubation on a shaker at 37°C, they were washed in HBSS containing 1 mM DTT (Sigma). Biopsies were blotted, weighed, and transferred to HEPES-buffered RPMI 1640 containing 20 mM HEPES, 1 mM DTT, penicillin/streptomycin (100 U/ml) (complete medium). They were incubated at 37°C in complete medium containing 1 mg/ml FITC-dextran. The EDTA and washing steps were repeated until no more epithelium was shed into the medium.

Isolation of lamina propria mononuclear cells (LPMC)

Two methods were used. In the first, fresh tissue was digested with 1 mg/ml collagenase D (Roche Molecular Products, Basel, Switzerland) in HEPES-buffered RPMI 1640 containing 20 μg/ml DNease I (Roche Molecular Products) and 2% FCS. Digestion at 37°C on a shaker was continued until visual inspection indicated that dissociation was complete. Typical this required an incubation of 90–180 min. Mononuclear cells were separated (650 × g, 20 min, room temperature) on Ficoll-Paque (Amer sham Pharmacia, Aylesbury, U.K.) and washed in complete medium. In some experiments cells isolated by collagenase digestion were cultured overnight at 1.5 × 10^6/ml in complete medium; in some cases cells were labeled with 1 mg/ml FITC-dextran (Sigma; 30 min, 37°C) and washed three times before culture.

In the second method, cells migrating from cultured biopsies were recovered as described by Mahida and colleagues (18). Each biopsy was placed in 0.5 ml of complete medium in a well of a 24-well culture plate (Falcon) and incubated for 24 h at 37°C in 5% CO₂. Migrating cells were recovered by centrifugation, and the biopsy was discarded or, in some experiments, cultured for one or more additional periods of 24 h.

Table I. Patient details

<table>
<thead>
<tr>
<th></th>
<th>CD</th>
<th>UC</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>24</td>
<td>14</td>
<td>22</td>
</tr>
<tr>
<td>Median age</td>
<td>33</td>
<td>43</td>
<td>55</td>
</tr>
<tr>
<td>Range</td>
<td>20–63</td>
<td>23–54</td>
<td>28–85</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>13/11</td>
<td>8/6</td>
<td>6/16</td>
</tr>
<tr>
<td>Disease activity</td>
<td>Quescent/Normal</td>
<td>Colon</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mild</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Moderate</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Severe</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fistula</td>
<td>3</td>
</tr>
<tr>
<td>Current treatment</td>
<td>Steroids</td>
<td>11</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5 ASA</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Azathioprine</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>No medication</td>
<td>6</td>
</tr>
</tbody>
</table>

Antibodies

Abs to HLA-DR (clone L243), CD3 (SK7), CD14 (M4p9), CD16 (B73.1), CD19 (4G7), CD34 (8G12), and CD62L (SK11) were purchased from BD Biosciences (San Jose, CA). Those to CD40 (LOB76), CD80 (DAL-1 or BB-1), CD86 (BU63), and were obtained from Serotec (Oxford, U.K.), and Abs to CD25 (ACT-1) and CD11c (KB90) were purchased from Dako (Carpenteria, CA). Anti-CD83 (HB15a) was purchased from Coulter Immunotech (Hialeah, FL), and anti-CD1a (IK-B5) was obtained from ImmunonKontact (Abingdon, U.K.). Anti-CD74 (M-B741) was purchased from BD Pharmingen (San Diego, CA). Isotype-matched controls were obtained from the same manufacturers.

Flow cytometry

Ab labeling was performed in PBS supplemented with 1 mM EDTA and 0.02% sodium azide (FACS buffer). FCS was added at 15% during the labeling steps and at 2% during the washing stages. Data were acquired using a FACSscan flow cytometer and were analyzed using CellQuest software (BD Biosciences). Dead cell and debris were excluded on the basis of light scatter. Using multicolor analysis, HLA-DR⁺ cells were divided into lin⁻ cells, which stained with a cocktail of Abs to T cells (CD3), B cells (CD19), NK cells (CD16), monocytes (CD14), and stem cells (CD34), and into lin⁺ cells, which did not stain with this mixture. Absolute cell counts were obtained by simultaneous acquisition of Flow-Count fluorospheres (Coulter Immunotech), and levels of cell surface marker expression on gated populations were determined by geometric mean fluorescence intensity (MFI) with subtraction of values for isotype-matched controls as appropriate.

Electron microscopy

Cells were fixed in 3% glutaraldehyde in 0.1 M PBS (pH 7.4) at room temperature for 2 h and postfixed in 1% osmium tetroxide for 1 h at room temperature. The cells were washed overnight in distilled water. Block staining was then performed with 2% aqueous uranyl acetate in water for 4 h, followed by washing and dehydration in graded acetone. The samples were then infiltrated with araldite overnight, embedded, and cured at 65°C for 18 h. The sections were stained with lead citrate and examined under a JEOL 1200 EX transmission electron microscope (Peabody, MA).

Endocytosis assay

Endocytic activity was assessed by measuring uptake of the fluid phase marker FITC-dextran (Sigma). Thirty thousand to 50,000 LPMC were incubated at 37°C in complete medium containing 1 mg/ml FITC-dextran. Control incubations were performed at 4°C. At various time points incubation was stopped by transfer of the cells to ice-cold FACS buffer, and the cells were labeled and subjected to flow cytometry as described above.

Enrichment of DC

DC were enriched or depleted from the "walk-out" LPMC population obtained from resection specimens (insufficient numbers were obtained to perform separations on cells obtained from biopsies). For negative selection of DC, LPMC were labeled with a cocktail of FITC-conjugated Abs to CD3, CD14, CD16, CD19, and CD34; for negative selection of an HLA-DR⁺ non-DC population, cells were labeled with a cocktail of FITC-conjugated Abs to CD3, CD16, CD19, CD34, and CD83. All labelings were performed at the use of a flow restrictor. Using these procedures DC were enriched ~10-fold and depleted ~4-fold, comprising >89% or <25% of the recovered HLA-DR⁺ cells, respectively.

MLR

MLR were performed using a 20-μl hanging drop culture system (19). Irradiated (1,800 cGy) stimulator cells (LPMC or enriched populations) were divided into 1 ml hanging drop culture system (19).
were added at between 625 and 5,000 cells to the wells of Terasaki plates containing 25,000 PBMC separated from the blood of a healthy control by centrifugation over Ficoll-Paque (described above). On the fifth day of culture, at 37°C in 5% CO₂, wells were pulsed with [3H]thymidine (1 μg/ml; sp act., 2 Ci/mmol) and, after 2 h, harvested by blotting onto filter paper. Incorporation of [3H]thymidine was assayed by liquid scintillation counting.

Statistical analysis
Two-tailed t tests were used to compare cell proportions and cell surface marker expression. DC numbers were compared using the nonparametric Mann-Whitney-Wilcoxon test. Values of p < 0.05 were regarded as significant.

Results
Identification of colonic DC
Leukocyte preparations were obtained from colonic biopsies by immediate enzymatic digestion and by harvesting cells migrating out of cultured tissue. These populations were termed day 0 (d0) collagenase and d1 walk-out populations, respectively. Multicolor flow cytometry permitted the identification of cell populations within this mixture despite the presence of autofluorescent cells that characterize tissue-derived populations (Figs. 1 and 2). Lin⁺ and Lin⁻ populations were identified among the HLA-DR⁺ cells in both d1 walk-out and d0 collagenase cells (Fig. 1). In all walk-out samples the HLA-DR⁻Lin⁻ cells were exclusively CD11c⁺ (Fig. 1A). This was also true for many of the d0 collagenase-digested samples (Fig. 1B), but in some samples a CD11c⁻ population was also present (Fig. 1C). In such cases, both the CD11c⁺ and CD11c⁻ populations were large granular cells (data not shown), but the former expressed higher levels of HLA-DR⁺ (Fig. 1), allowing the two populations to be discriminated in subsequent analyses (Fig. 1).

Fig. 2 illustrates further analysis of the staining obtained with d0 collagenase cells. The HLA-DR⁺ lin⁻ and HLA-DR⁺ lin⁻ populations were located within the gate of large cells, but were absent among cells with the light scatter properties of lymphocytes (Fig. 2A). Isotype controls confirmed the specificity of the labeling (Fig. 2B). Labeling with individual components of the lineage cocktail indicated that cocktail staining of the HLA-DR⁺ lin⁻ population could be attributed to anti-CD14, because in the absence of this Ab.

**FIGURE 1.** Identification of colonic DC in cells obtained on d1 by the walk-out method (A) and on d0 by collagenase digestion (B and C). Flow cytometry demonstrates that all HLA-DR⁻lin⁻ lamina propria cells in the population that migrates out of culture biopsies express CD11c (A). HLA-DR⁻lin⁻ colonic cells isolated by collagenase digestion of most fresh tissue samples were also CD11c⁺ (B). However, in some enzyme-digested samples, a CD11c⁻ HLA-DR⁺ lin⁻ population was also present (C). In these cases, the CD11c⁺ and CD11c⁻ subpopulations can be separated on the basis of the level of HLA-DR expression.
the majority of cells dropped out of the HLA-DR\textsuperscript{+}Lin\textsuperscript{2} region (Fig. 2C; compare CD14 staining with that of the other lineage cocktail components). CD3\textsuperscript{+} and CD19\textsuperscript{+} cells, lacking HLA-DR, could also be identified within the large cell gate, as could a population of CD34\textsuperscript{+} cells (Fig. 2C), which immunohistology suggested were vascular endothelial cells (data not shown).

Analysis of the kinetics of walk-out cultures demonstrated that the majority (≥80%) of DC were recovered during the first 24 h of culture and that leukocytes obtained at this time point were relatively enriched for DC (2.2% of recovered cells at 0–24 h, 1.1% at 24–48 h, and 0.3% at 48–72 h), suggesting that DC migration is more rapid than that of other cell populations. Electron microscopy of walk-out cells confirmed the presence of DC (Fig. 3). DC are heterogeneous by electron microscopy and have been classified into types 1, 2, and 3 (20). Type 1 DC are smaller, irregularly shaped cells with small cell surface projections and heterochromatic nuclei in which the chromatin is present in a thick band around the margin and in small condensed areas. Type 2 DC are larger with few projections and have a euchromatic nucleus with chromatin disaggregated and present as a thin band at the nuclear margin. Type 3 DC have a veiled appearance and euchromatic nuclei. The significance of these different morphological forms is not yet clear, but all three were present within the walk-out population. We did not observe Birbeck granules in the walk-out colonic DC.

HLA-DR\textsuperscript{ Lin}\textsuperscript{d1} walk-out cells, but not the double-positive population, stimulated potent primary T cell responses (Fig. 4), supporting the identification of the Lin\textsuperscript{−} and Lin\textsuperscript{+} subpopulations as DC and monocyte/macrophages, respectively.

Phenotype of colonic DC

The expression of additional markers on the surface of d0 and d1 colonic HLA-DR\textsuperscript{+}Lin\textsuperscript{−} cells was assessed using three-color flow cytometry. Where necessary, CD11c\textsuperscript{+} and CD11c\textsuperscript{−} subpopulations were separated on the basis of level of HLA-DR expression as shown in Fig. 1. Fig. 5 shows representative data for this type of FACS experiment. The DC obtained after the culture of biopsies for 24 h had the phenotype of mature cells consistent with their potent ability to stimulate an allo-MLR. They were CD83 positive and expressed high levels of CD80, CD86, CD40, and CD25. In contrast, the CD11c\textsuperscript{−} d0 population had a phenotype consistent with immature DC, i.e., CD40\textsuperscript{+} but expressing low levels of CD83 and CD86 with little or no CD80 and CD25.

Of the cell surface markers examined, the CD11c\textsuperscript{−} population within the HLA-DR\textsuperscript{+}Lin\textsuperscript{−} gate expressed only CD40, and this was at a low level (data not shown). This population did not express CD62L (data not shown), suggesting that they are not equivalent to the CD11c\textsuperscript{−} DC subpopulation identifiable in peripheral blood, as high level expression of CD62L is characteristic of these blood cells (21). In the absence of a definitive identification of these CD11c\textsuperscript{−}HLA-DR\textsuperscript{+}Lin\textsuperscript{−} gut cells, the quantitative phenotypic and
functional analysis presented below is restricted to the CD11c^+ population.

To rule out the possibility that exposure to collagenase/DNase may remove markers from the surface of DC, mature DC were generated by culture of peripheral blood mononuclear cells, incubated with the enzyme mixture, and subsequently labeled. Expression of CD86 and CD40 was unaffected by exposure to the collagenase mix (CD86, MFI of 115 without collagenase treatment vs 115 for treated cells; CD40, MFI of 47 without collagenase vs 43 for treated cells). CD11c was expressed at high levels after enzymatic treatment (MFI = 149), although levels were reduced compared with those in untreated cells (MFI = 244). CD83 was also slightly affected by collagenase treatment (MFI = 143 for untreated cells; MFI = 71 for treated cells), but levels were still more typical of d1 walk-out colonic DC than of d0 colonic DC. These experiments also failed to show any evidence of a toxic affect of enzyme treatment on mature DC.

**Endocytic activity of colonic DC**

That d0 and d1 CD11c^+HLA-DR^-lin- are immature and mature DC populations, respectively, is supported by their endocytic activities (Fig. 6). The d0 cells were endocytically active, with a level of FITC-dextran uptake intermediate between that of gut lymphocytes and that of HLA-DR^-lin- monocyte/macrophages. Endocytosis by d1 walk-out CD11c^-HLA-DR^-lin- cells was less than that by their d0 counterparts (Fig. 6), consistent with increased maturity. Those DC remaining in the tissue also maintained their
endocytic activity, suggesting that maturation and migration go hand-in-hand.

**DC number in inflamed and noninflamed intestines**

On the basis that cell recoveries by the d0 collagenase technique were likely to best reflect the numbers present in the tissue, we used this method to measure DC numbers. Overall, CD11c+ DC comprised 0.59 ± 0.35% of the colonic cell population (n = 52). This was equivalent to a median of 114 CD11c+ DC/mg of tissue (n = 51; interquartile range, 78–220). Proportions of DC did not differ significantly between controls and CD patients or between inflamed and noninflamed CD tissue (the means were 0.62, 0.57, and 0.61% for control, noninflamed CD tissue, and inflamed CD tissue, respectively). The median number of DC isolated was higher from inflamed tissue (146/mg of tissue compared with 115 for both control and noninflamed CD tissue; Fig. 7A), but this difference did not reach statistical significance.

The proportion of cells identified as CD11c+ HLA-DR+ lin- was more variable, ranging from 0 to 27.9%. As a result of the occasional sample with a very high proportion of these cells, the observed differences among controls, noninflamed CD tissue, and inflamed CD tissue (mean, 1.6, 3.3, and 4.7%, respectively) did not reach statistical significance. However, a high frequency of CD11c+ HLA-DR+ lin- cells was more common in CD patients, 10 of 21 of whom had >2% CD11c+ HLA-DR+ lin- cells, than in controls (1 of 25 patients). The proportion of HLA-DR+ lin- cells was significantly less in CD (1.9 ± 1.5%; n = 23) than in controls (4.2 ± 2.7%; n = 22), but did not significantly differ between inflamed and noninflamed tissue.

**Phenotype of DC from healthy and IBD colonic tissue**

When levels of CD80, CD86, CD25, and CD83 were compared, CD11c+ DC from IBD patients and controls appeared to be at a similar stage of maturation. For all four markers, levels were significantly (p < 0.001) higher on d1 walk-out cells than on the d0 population (Fig. 7B). CD80 and CD25 were almost completely absent on d0 cells, whereas CD86 and CD83 were expressed at low and variable levels. A similar pattern of results was obtained with two different CD80-reactive Abs, DAL-1 and BB-1, although staining of the d1 cells with BB-1 tended to be brighter (data not shown). This was not due to BB-1’s reported cross-reactivity with...
invariant chain (CD74) (22) as the d1 walk-out population did not stain with anti-CD74 (data not shown).

Maturation of colonic DC

To address the question of whether freshly isolate DC have the potential to undergo maturation, we examined the phenotype of cells prepared on d0 by collagenase digestion and then cultured overnight. Cells cultured overnight in this way up-regulated CD40, CD80, CD86, and CD25 compared with the starting d0 population, confirming a potential to undergo maturation. However, all markers were expressed at higher levels on DC migrating out of biopsies in parallel cultures (Fig. 8). Furthermore, nonmigrating DC obtained on d1 by collagenase digestion of the cultured biopsy expressed lower levels of these cell surface markers than DC that had migrated out of the tissue (data not shown).

Differentiation of colonic DC from HLA-DR$^+$ lin$^+$ precursors

In four of five experiments in which cells were compared on d0 and after overnight incubation, an increase (30–70%) in the recovered number of CD11c$^+$ DC was noted, raising the possibility that some cells initially outside the HLA-DR$^+$ lin$^-$ gate acquire this phenotype during culture. A likely candidate for the source of such cells is the HLA-DR$^+$ lin$^+$ population, as the differentiation

FIGURE 7.  A, Numbers of CD11c$^+$ DC isolated from control and CD tissue. The filled symbol (•) shows the median number of cells; the box is the interquartile range. B, d1, but not d0, CD11c$^+$ DC express high levels of CD80, CD86, CD25, and CD83. Geometric mean fluorescence intensity was used in three-color flow cytometry to compare levels of these markers on cells from patients with CD, active ulcerative colitis, or quiescent ulcerative colitis and cells from individuals under investigation for noninflammatory conditions. Cells were obtained from both inflamed and noninflamed tissue. Expression of all three markers was absent or low on d0 cells even when the tissue was inflamed.

FIGURE 8. Overnight culture of d0 collagenase cells yields DC with a phenotype intermediate between that of the starting population and that of d1 walk-out cells. Net MFI for CD11c$^+$ DC was calculated for the markers indicated by subtraction of the isotype control binding. Relative MFI was calculated by normalizing the data to the values obtained for the walk-out population.
of CD14+ cells into DC is well established. To address this issue we took advantage of the differential uptake of FITC-dextran by HLA-DR+lin− and HLA-DR+lin+ cells (Figs. 6 and 9). Lamina propria cells were isolated by collagenase digestion, labeled with FITC-dextran, washed, and analyzed immediately or following overnight culture. As previously described, the HLA-DR+lin− population labeled strongly with FITC-dextran, and this was maintained during the overnight culture (Fig. 9). The DC population on d0 took up a smaller amount of FITC-dextran, and this was clearly distinguishable from the amount present in either fresh or cultured HLA-DR+lin− populations. After culture a population of DC displayed a level of FITC labeling characteristic of the HLA-DR+lin− population (Fig. 9). Lymphocytes displayed little or no labeling before or after culture (data not shown). These results are consistent with a differentiation of FITC-dextran-bearing HLA-DR+lin+ cells into DC during overnight culture and provide further support for the importance of a pathway of DC maturation that proceeds via a monocyte intermediate.

**Discussion**

In this study DC have been identified and characterized for the first time in leukocyte preparations obtained from small amounts of human colonic and rectal tissue. Cells freshly isolated from the lamina propria contained a population of CD11c+ DC with the phenotype and functional properties of immature cells. The same mucosal tissue also contained cells capable of developing into mature CD11c+ DC upon culture of the tissue. The use of multicolor analysis enabled the identification of DC with confidence despite problems such as high levels of autofluorescence that are inherent in flow cytometry of cells extracted from tissues. Using combinations of enzymatic digestion and a walk-out culture system, the relationship among cell surface phenotype, maturational status, and migratory activity has been examined.

The relationship between the freshly isolated and mature DC populations remains to be determined, but the simplest view is that the population identified on d0 matures into the population identified on d1. This would parallel the situation that occurs with Langerhans’ cells in skin explants; immature epidermal Langerhans cells migrate out of the skin via the dermis and in doing so begin to mature (23, 24). However, cells differentiating into mature DC from other populations, such as the HLA-DR+Lin− population, may also contribute. Following labeling of this latter population with FITC-dextran and overnight culture, cells appear within the DC (HLA-DR+Lin−) gate with a level of FITC labeling characteristic of the HLA-DR+Lin− population. These findings are suggestive of differentiation of HLA-DR+Lin− cells into DC, an interpretation favored by the increase in DC numbers during culture. However, the possibility that high levels of FITC-dextran are transferred to a subpopulation of DC or that maturation somehow influences FITC fluorescence cannot currently be excluded. Nonetheless, the HLA-DR+Lin− cells are CD14+, and there is abundant evidence that CD14+ cells can differentiate into DC in vitro and in vivo, particularly under conditions of inflammation (25–28). Thirdly, the CD11c+ DC population could potentially differentiate from the CD11c− HLA-DR+Lin− population, although there is no evidence for interconversion of CD11c+ and CD11c− DC populations in peripheral blood (21). The CD11c− HLA-DR+Lin− population labeled with few of the other Abs tested and its nature remains to be determined. It may correspond to the CD11c− HLA-DRlow CD14+ macrophage population recently isolated from normal colonic mucosa (29) or to the population with DC-like properties that was enriched from lamina propria by Pavić and colleagues and expressed little or no CD11c (15). If they constitute a subpopulation of DC, they differ from CD11c− peripheral blood DC in their lack of CD123 and CD62L expression (A. J. Stagg, S. J. Bell, R. Rigby, M. A. Kamm, and S. C. Knight, unpublished observations) (21). Importantly, the lack of these markers on HLA-DR+Lin− cells in the preparations from colonic mucosa suggests that there is minimal contribution by contaminating peripheral blood.

It is currently unclear whether the DC (or precursors of DC) migrate out of the biopsy through pores in the basement membrane, as occurs for other leukocytes in similar culture systems (18), or whether they exit in the opposite direction as if migrating to draining lymphoid tissue (30). After 24 h in culture these walk-out DC express uniformly high levels of maturation-dependent markers. This maturation appears more rapid than that reported for Langerhans cells migrating out of murine skin tissue (23) or for human Langerhans’ cells in culture (31, 32). This discrepancy may reflect a history of increased Ag exposure that is characteristic of the mucosal population. The walk-out culture system may provide a useful model in which the roles of cytokines, chemokines, and other molecules in the maturation of gut DC can be dissected. The supernatant of cultured colonic biopsies is rich in proinflammatory cytokines (R. Rigby, S. J. Bell, M. A. Kamm, S. C. Knight, and A. J. Stagg, unpublished observations). Although CD80, CD86, CD25, and CD40 are up-regulated during overnight culture of d0 collagenase DC, they do not reach the levels expressed on walk-out DC. This incomplete maturation may result from rather non-specific effects of tissue dissociation, but it could also indicate an absence of important signals provided during migration through the tissue and across the basement membrane. There is evidence that DC can receive maturational signals during migration across endothelial tissue (27).

Immunohistological studies have described disease-associated changes in APC populations in IBD (12, 13, 33–36), including evidence for activation in early aphthoid lesion in CD. However, the contributions of these cells in general and of DC in particular to the immune dysregulation underlying these conditions (37) are not known. Abnormality of DC function has been reported in one transgenic model of colitis (38). In experimental models of autoimmune disease, injection of Ag-bearing DC can induce inflammation (39–41) and the neogenesis of organized lymphoid structures (41). Sallusto and Lanzavecchia have recently suggested that the failure of mature DC to leave peripheral tissue and migrate to lymph nodes could underlie such processes in some inflammatory diseases (17). Mature DC remaining in the tissue could recruit...
recently activated T cells and participate in rounds of mutual stimulation with these cells with the accompanying production of growth and differentiation factors. In postchlamydial reactive arthritis we found DC bearing chlamydial Ags localized within inflamed joints (42). If the processes proposed by Sallusto and Lan-zavecchia occur in CD, mature DC should be identifiable within inflamed lamina propria. We found no evidence for such mature DC, expressing high levels of costimulatory and other maturation-dependent markers, among the cells extracted immediately from colonic tissue. This was the case regardless of whether the tissue was inflamed. Recently, DC identified in an immunohistochemical study as mature on the basis of CD86 expression have been identified in the gastric mucosa in autoimmune gastritis (43), suggesting that nonmigrating mature DC might contribute to other forms of intestinal inflammation.

Although fully mature DC were not found in cells from IBD colon, it is possible that subtle alterations in functionally important DC cell surface molecules would be revealed in a larger study or that other activities of DC, such as the production of soluble mediators, contribute to local inflammatory reactions. It could be argued that mature DC are present in the inflamed lamina propria, but are not extracted by the methods used, are lost during the separation procedure, or have their phenotype altered by exposure to the digestion enzymes. In other contexts collagenase treatment has proved effective in extracting tightly bound DC subpopulations (44). Control experiments on mature DC prepared from peripheral blood suggested that the low expression of DC maturation markers on d0 colonic DC is unlikely to be due to a deleterious effect of enzymatic treatment. Furthermore, positive control markers, including CD45 and MHC class I, are expressed at high level on gut DC obtained by enzymatic digestion.

We found no statistically significant evidence for an increase in DC numbers in inflamed colonic tissue, although median values were higher. This may simply reflect the imprecise nature of quantifying enzyme-extracted cells. Alternatively, this initially surprising finding may be explained if the DC that are extracted from the tissue represent the net balance between recruitment of cells and migration to the draining lymph nodes. Animal studies suggest that inflammatory stimuli may increase both the recruitment and the rate of exit of mucosal DC (1, 45, 46). Thus, the numbers of DC resident in the tissue at a given time need not be obviously increased. It follows that gut DC may mediate their immunoregulatory effects in the draining lymph nodes, and it is here that their influence in IBD may be exerted. We hope to address these questions in future studies.

There is direct evidence for recruitment of CD14+ monocytes into mucosal tissue in CD (47), and as discussed above, these cells may represent an important pool of DC precursors. Our finding of a reduced proportion of HLA-DR+lin- (almost all of which are CD14+) in CD tissue compared with control tissue could reflect an increase in in vivo differentiation into DC in the disease state.

Collectively, these data illustrate the feasibility of using flow cytometry to identify and characterize DC in cell populations isolated from small amounts of human gut tissue. For the first time the phenotype and maturation and migratory activities of DC from both healthy and IBD gut tissue have been determined.

References


