Surfactant protein A modulates the differentiation of murine bone marrow-derived dendritic cells

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Brinker, Karen G., Hollie Garner, and Jo Rae Wright. Surfactant protein A modulates the differentiation of murine bone marrow-derived dendritic cells. Am J Physiol Lung Cell Mol Physiol 284: L232–L241, 2003.—Surfactant protein A (SP-A) is an innate immune molecule that regulates pathogen clearance and lung inflammation. SP-A modulates innate immune functions such as phagocytosis, cytokine production, and chemotaxis; however, little is known about regulation of adaptive immunity by SP-A. Dendritic cells (DCs) are the most potent antigen-presenting cell with the unique capacity to activate naïve T cells and initiate adaptive immunity. The goal of this study was to test the hypothesis that SP-A regulates the differentiation of immature DCs into potent T cell stimulators. The data show that incubation of immature DCs for 24 h with SP-A inhibits basal- and LPS-mediated expression of major histocompatibility complex class II and CD86. Stimulation of immature DCs by SP-A also inhibits the allostimulation of T cells, enhances dextran endocytosis, and alters DC chemotaxis toward RANTES and secondary lymphoid tissue chemokine. The effects on DC phenotype and function are similar for the proteolytic activity and the collectin family includes surfactant protein A (SP-A), which is also a collectin. The structurally similar complement component 1q (C1q) is also a defense collagen. The collectins generally function in innate immunity by binding to a variety of carbohydrates abundant on the surfaces of microorganisms and by interacting with immune cells to promote phagocytosis (16). Specifically, SP-A has also been shown to play a role in regulating lung inflammation by modulating other immune functions including chemotaxis (61), cytokines...
production (39), complement activation (58), and T cell proliferation (3, 6). Mice made deficient in SP-A by targeted disruption of the gene are more susceptible to bacterial and viral infection (33, 35–37) and LPS-mediated inflammation (4), demonstrating the importance of SP-A in regulating immune responses in the lung.

Because SP-A has an established role in affecting the functions of a variety of immune cells, the goal of the present study was to test the hypothesis that SP-A modulates the activity of DCs. We investigated whether SP-A and other defense collagens influence the phenotypic and functional differentiation of immature DCs to become potent T cell stimulators. We show that both SP-A and C1q, but not SP-D, negatively regulate the maturation of DCs by inhibiting the expression of major histocompatibility complex (MHC) class II and CD86, resulting in a decreased T cell allostimulatory ability. Furthermore, stimulation of immature DCs by both SP-A and C1q enhances their endocytic ability and modulates their chemotactic responses to known chemokines. Our results establish a role for defense collagens in controlling adaptive immune responses through interactions with DCs.

**METHODS**

**Materials.** Media, balanced salt solutions, antibiotics, and 2-mercaptoethanol were purchased from Life Technologies (Gaithersburg, MD). Heat-inactivated fetal bovine serum was purchased from HyClone (Logan, UT). All other chemicals and reagents were obtained from Sigma (St. Louis, MO), except where noted.

**Animals.** Male C57BL/6 or female BALB/c mice were obtained from Charles River Laboratory (Raleigh, NC) and housed in pathogen-free conditions. All mice were between 6 and 8 wk of age.

**Antibodies.** Rat anti-mouse CD16/CD32 (Fc block), biotin and fluorescein isothiocyanate (FITC) rat anti-mouse Ly-6G (GR-1), phycoerythrin (PE) mouse anti-mouse I-A^k^ (MHC class II), PE mouse anti-mouse H-2K^a^, PE rat anti-mouse CD86, FITC hamster anti-mouse CD80, FITC hamster anti-mouse CD40, FITC hamster anti-mouse CD54, PE hamster anti-mouse CD11c, and all relevant isotype controls were purchased from Pharmingen (San Diego, CA).

**Protein isolation.** Human SP-A was purified from patient BAL supernatants via butanol extraction as previously described (39). SP-A was treated with polymyxin agaroate to reduce endotoxin contamination, and all SP-A preparations were tested for endotoxin via Limulus amebocyte lysate assay QCL-1000 (BioWhittaker, Walkersville, MD) and were found to have $<0.5$ pg endotoxin/$\mu$g SP-A. Recombinant rat SP-D was purified by maltose affinity chromatography from the medium supernatant of cultured Chinese hamster ovary cells expressing a full-length rat SP-D cDNA clone as previously described (15). The SP-D preparations were tested for endotoxin and were found to have $<0.5$ pg endotoxin/$\mu$g SP-D.

**Fluorescence-activated cell sorting characterization for maturation markers.** BMDCs were cultured in 24-well plates at $5 \times 10^5$ cells/ml in macrophage-serum-free medium (Life Technologies) containing 10 mM HEPES, 50 $\mu$M 2-mercaptoethanol, and 100 U/ml penicillin-streptomycin. The cells were stimulated in the presence or absence of 100 ng/ml Escherichia coli LPS (serotype O55:B5 or L2352) and various concentrations of SP-A, SP-D, or C1q for 24 h. After 24 h, nonadherent cells were harvested and suspended to $1 \times 10^6$ cells/ml in fluorescence-activated cell sorting (FACS) buffer [Dulbecco’s phosphate-buffered saline (DPBS) containing 1% BSA]. The cells were seeded into a V-bottomed 96-well plate at 100 $\mu$l/well and Fc receptor blocked using purified anti-CD16/CD32 antibody followed by incubation with directly conjugated antibodies (0.5 $\mu$g) for 30 min on ice. The samples were washed twice, suspended in 1% formaldehyde solution, and analyzed by flow cytometry. In preliminary experiments, SP-A formed aggregates during the overnight culture that spun down with the BMDCs. The SP-A aggregates displayed a distinct forward-angle light scatter/ side-angle light scatter (FSC/SSC) profile compared with the BMDCs in FACS analysis; however, the nucleic acid dye 7-amino-actinomycin D (7-AAD; PharMingen) was added to fixed BMDCs at 5 $\mu$g/sample to ensure that only cells were being analyzed. Samples were then analyzed by dual gating for FSC/SSC characteristics and expression of 7-AAD.

**Allostimulation assays.** Allostimulation assays were performed as previously described (14, 28). Immature BMDCs were cultured for 24 h in the presence of LPS (O55: B5), SP-A, or C1q for 24 h were harvested at day 7, washed, fixed with 0.5% paraformaldehyde, and plated at threefold serial dilutions in round-bottomed plates. The stimulator BMDCs were incubated with 5 $\times 10^4$ cells/well of splenic CD4^+^ T cells [purified from BALB/c mice using MACS with CD4 microbeads (Miltenyi Biotec)] for 4 days. Proliferation was measured by pulsing with 1 $\mu$Ci/well ^3^H thymidine for the last 18 h. Cells were harvested with a Combi cell harvester (Skatron, Sterling, VA), and incorporated ^3^H thymidine was measured with a beta scintillation counter.

**Chemotaxis.** Chemotaxis assays were performed as described previously (53, 61). Briefly, 50 $\mu$l of cells [immature BMDCs stimulated for 24 h in the presence or absence of LPS (O55: B5), SP-A, or C1q] suspended at $1 \times 10^5$ cells/ml in Gey’s balanced salt solution (GBSS) containing 1% BSA were placed in the upper wells of a 48-well microchemotaxis chamber (Neuro Probe, Cabin John, MD). The lower chambers contained 27 $\mu$l (100 ng/ml final concentration) of the chemo-
kines regulated on activation, normal T cell expressed, and secreted (RANTES) or secondary lymphoid-tissue chemokine (SLC) (obtained from PeproTech, Rocky Hill, NJ) or GBSS buffer only, in triplicate. A polyvinylpyrrolidone-free polycarbonate filter with 5 μm pores was placed between the chemottractants and cells. The chamber was incubated at 37°C with 5% CO₂ for 90 min. Nonmigrating cells were scraped from the upper surface, and the migrating cells were stained with the Hemacolor differential blood stain. Cells that migrated through the filter were counted in 10 randomly selected oil-immersion fields/well at ×1,000 magnification.

Endocytosis assay. Endocytosis was quantitated by assaying for uptake of FITC-dextran as described (45). Briefly, immature BMDCs stimulated in the presence or absence of LPS (O55:B5), SP-A, or C1q for 24 h were suspended to 1 × 10⁶ cells/ml in DPBS containing 1% BSA. DCs (2 × 10⁶) were pulsed with FITC-dextran (40,000 mol wt; Molecular Probes, Eugene, OR) at a concentration of 1 mg/ml for 1 h at 37°C. In some experiments, 25 μg/ml exogenous SP-A or C1q was added during the pulsing incubation. Cells were washed two times, fixed in 1% formaldehyde, and analyzed by FACS.

FACS. FACS was performed at the Duke Comprehensive Cancer Center Flow Cytometry Facility. Samples (~10,000 cells/treatment) were analyzed for fluorescence per cell at the selected wavelength. Data were analyzed by CELLQUEST or FLOWJO software.

Statistics. Data were compared by two-tailed paired Student's t-test or analysis of variance with Tukey where appropriate. Values were considered significant at P < 0.05.

RESULTS

Differentiation of DCs in vitro. Murine DCs were differentiated in vitro from bone marrow progenitor cells incubated with GM-CSF. At day 6 of culture, the nonadherent population of cells was enriched (70%) for immature DCs with a phenotype consisting predominantly of CD11c⁺, MHC class II intermediate, CD80low, CD86low, and GR-1neg as previously reported (12).

SP-A regulates phenotypic changes of DC. Nonadherent day 6 immature BMDCs were used in experiments to test the effects of SP-A on DC differentiation, using an approach similar to other studies (31). SP-A was incubated with day 6 immature BMDCs for 24 h in the absence of serum or GM-CSF to minimize the effects of exogenous factors on DC maturation (31, 44). The results in Fig. 2 show that incubation of day 6 BMDCs in medium only (untreated) for 24 h resulted in a high basal-level expression of MHC class II and CD86 as expected, since DCs can be partially stimulated by mechanical manipulation during transfer between culture plates (19). Interestingly, this basal-level expression of MHC class II and CD86 was markedly reduced (up to 40–60% reduction) by treatment of BMDCs with SP-A as assessed by the mean fluorescence intensity (MF) of the entire population of cells analyzed. Interestingly, the reduction in the MF seems to be due to a decreased percentage of cells that become high expressers for either MHC class II or CD86 in the presence of SP-A (Fig. 2B). The inhibition of MHC class II (Fig. 3A) and CD86 (Fig. 3B) expression by SP-A was dose dependent from 10 to 120 μg/ml of SP-A, concentrations estimated to be within physiological ranges in the lung (59). However, SP-A had no significant effect on the expression of the DC-restricted antigen CD11c (Fig. 2A). As expected, treatment of immature BMDCs with LPS resulted in an upregulation of both MHC class II and CD86 compared with untreated cells, which was also inhibited by SP-A (Fig. 2, A and B). The effect of SP-A on LPS-induced maturation was similar using either a rough (LCD25) or smooth (O55:B5) serotype of LPS (data not shown). Likewise, the effect of SP-A on DC differentiation was independent of the maturation stimulus, since SP-A significantly inhibited expression of MHC class II and CD86 in GM-CSF-supplemented medium (data not shown).

To determine whether SP-A mediated additional changes in the phenotype of BMDCs, we assessed the
expression of other DC regulatory molecules. Table 1 shows that the effects of SP-A on basal and LPS-induced stimulation of BMDCs were most pronounced for MHC class II and CD86 expression, with lesser effects on the expression of MHC class I, CD54, and CD80 expression. Based on mean fluorescence values, no significant difference was observed for CD40 expression on BMDCs after incubation with SP-A.

Modulation of maturation marker expression by SP-A is shared by the structurally similar protein C1q.

To examine whether the regulation of DC differentiation was specific to SP-A, other members of the collec-

Table 1. Effect of SP-A on the expression of maturation markers on BMDCs

<table>
<thead>
<tr>
<th>Maturation Marker</th>
<th>Untreated</th>
<th>SP-A</th>
<th>LPS</th>
<th>LPS + SP-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>MHC class II</td>
<td>745 ± 77</td>
<td>541 ± 64*</td>
<td>1057 ± 101†</td>
<td>793 ± 111*</td>
</tr>
<tr>
<td>MHC class I</td>
<td>75 ± 18</td>
<td>55 ± 13</td>
<td>153 ± 25†</td>
<td>136 ± 25†</td>
</tr>
<tr>
<td>CD86</td>
<td>170 ± 19</td>
<td>99 ± 13*</td>
<td>404 ± 86†</td>
<td>269 ± 71†</td>
</tr>
<tr>
<td>CD80</td>
<td>73 ± 10</td>
<td>101 ± 10*</td>
<td>92 ± 16†</td>
<td>90 ± 11</td>
</tr>
<tr>
<td>CD40</td>
<td>78 ± 19</td>
<td>82 ± 14</td>
<td>78 ± 13</td>
<td>80 ± 12</td>
</tr>
<tr>
<td>CD54</td>
<td>96 ± 17</td>
<td>79 ± 13*</td>
<td>164 ± 29†</td>
<td>141 ± 22</td>
</tr>
</tbody>
</table>

Values are means ± SE. Immature bone marrow-derived dendritic cells (BMDCs) were incubated in the presence or absence of lipopolysaccharide (LPS; serotype O55:B5) or 40 μg/ml SP-A, for 24 h. Nonadherent BMDCs were collected and analyzed for expression of maturation markers by fluorescence-activated cell sorting (FACS). SP-A, surfactant protein A. The data shown are mean fluorescence values from n = 4 individual experiments; *P < 0.05 by a Student t-test comparing SP-A condition with untreated condition or LPS + SP-A condition with LPS condition; †P < 0.05 comparing LPS condition with untreated condition.
tin family and the structurally similar protein C1q were tested in experiments with BMDCs. Table 2 shows that, like SP-A, treatment of BMDCs with C1q, but not SP-D, potently altered the expression of DC regulatory molecules (up to 65% reduction) by inhibiting the expression of MHC class II and CD86. Interestingly, although SP-A slightly enhanced the expression of CD80 on BMDCs (P = 0.04), incubation of immature BMDCs with C1q for 24 h enhanced the expression of CD80 by greater than twofold. The effects of SP-A and C1q on DC marker expression were not due to toxicity induced by treatment of DCs with the proteins, since there were no differences in cell viabilities between treatment groups as assessed by trypan blue staining, averaging 85 ± 3, 90 ± 2, 91 ± 4, or 93 ± 2% viable for untreated, SP-A-, C1q-, or LPS-treated cells, respectively. Likewise, there were no differences in viabilities between treatment conditions as detected by staining for 7-AAD/annexin (data not shown).

SP-A and C1q inhibit the allostimulatory ability of DCs. The primary function of DCs to activate T cells is dependent on the expression of MHC and costimulatory molecules. To test the hypothesis that defense proteins, since there were no differences in cell viabilities between treatment groups as assessed by trypan blue staining, averaging 85 ± 3, 90 ± 2, 91 ± 4, or 93 ± 2% viable for untreated, SP-A-, C1q-, or LPS-treated cells, respectively. Likewise, there were no differences in viabilities between treatment conditions as detected by staining for 7-AAD/annexin (data not shown).

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**Table 2. Regulation of maturation marker expression by collectin-like proteins**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>CD86</th>
<th>CD80</th>
<th>MHC Class II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>LPS</td>
<td>309 ± 25*</td>
<td>162 ± 9*</td>
<td>160 ± 14*</td>
</tr>
<tr>
<td>SP-A</td>
<td>60 ± 4*</td>
<td>130 ± 12*</td>
<td>70 ± 3*</td>
</tr>
<tr>
<td>SP-D, 1 µg/ml</td>
<td>104 ± 1</td>
<td>99 ± 7</td>
<td>n.d.</td>
</tr>
<tr>
<td>SP-D, 5 µg/ml</td>
<td>100 ± 7</td>
<td>95 ± 7</td>
<td>n.d.</td>
</tr>
<tr>
<td>C1q</td>
<td>49 ± 6*</td>
<td>235 ± 30*</td>
<td>42 ± 3*</td>
</tr>
</tbody>
</table>

Immature BMDCs were incubated in the presence or absence of LPS (serotype LCD25), 40 µg/ml SP-A, 1 or 5 µg/ml SP-D, or 40 µg/ml C1q for 24 h. Nonadherent BMDCs were collected and were analyzed for expression of maturation markers by FACS. n.d., Not determined. The data are reported as the percent change in mean fluorescence compared with the untreated condition ± SE; n > 4 individual experiments; *P < 0.05 by a Student t-test comparing protein condition with untreated condition.

Fig. 4. SP-A and C1q inhibit BMDC allostimulation of T cells. Immature BMDCs were incubated in the presence or absence of 40 µg/ml SP-A or 40 µg/ml C1q for 24 h. Nonadherent BMDCs were collected, washed, fixed, and added to allogeneic CD4+ T cells for 4 days. For the SP-A control, 40 µg/ml SP-A was incubated in medium alone containing no cells for 24 h and was then added to BMDCs incubated in the absence of added protein for subsequent processing. Proliferation was measured by adding [3H]thymidine for the last 24 h. DC, dendritic cell. Data are reported as ± SE of counts per min (cpm) values from individual experiments; n > 3. *P < 0.05 comparing SP-A condition to untreated condition, †P < 0.05 comparing C1q condition to untreated condition.

**Table 3. Effect of SP-A on LPS-induced allostimulation**

<table>
<thead>
<tr>
<th>T Cell/DC Ratio</th>
<th>Untreated</th>
<th>LPS</th>
<th>LPS + SP-A</th>
</tr>
</thead>
<tbody>
<tr>
<td>2:1</td>
<td>2,387 ± 373</td>
<td>3,793 ± 563</td>
<td>2,915 ± 466*</td>
</tr>
<tr>
<td>14:1</td>
<td>2,416 ± 296</td>
<td>3,670 ± 546†</td>
<td>2,979 ± 424*</td>
</tr>
<tr>
<td>42:1</td>
<td>1,314 ± 90</td>
<td>2,520 ± 270†</td>
<td>2,110 ± 103</td>
</tr>
<tr>
<td>125:1</td>
<td>711 ± 75</td>
<td>1,253 ± 160†</td>
<td>908 ± 39</td>
</tr>
</tbody>
</table>

Immature BMDCs were incubated in the presence or absence of LPS (serotype O55:B5) or 40 µg/ml SP-A for 24 h. Nonadherent BMDCs were collected, washed, fixed, and incubated with allogeneic CD4+ T cells for 4 days. For the last 18 h, [3H]thymidine was added. The data are reported as counts per min ± SE of n > 3 experiments; *P < 0.05 by a Student t-test comparing LPS + SP-A condition with LPS condition or †P < 0.05 comparing LPS condition with untreated condition.
stimulate BMDCs to become more endocytic, we treated immature BMDCs in the presence or absence of LPS, SP-A, or C1q for 24 h followed by the addition of FITC-dextran to assess endocytosis. Figure 5 shows that untreated BMDCs showed a high basal level of endocytosis of FITC-dextran, which was significantly upregulated on treatment of BMDCs with SP-A or C1q. Endocytosis was reduced with BMDCs stimulated with LPS, although concurrent incubation of BMDCs with LPS in the presence of SP-A enhanced endocytosis to levels similar to the untreated condition. Like the MR, SP-A is a C-type lectin with similar carbohydrate binding specificities (11). Thus to ensure that any SP-A remaining after the 24-h incubation was not responsible for the enhanced uptake of FITC-dextran, we added 25 μg/ml SP-A to the untreated cells during the uptake reaction. Neither exogenously added SP-A nor C1q significantly increased the uptake of FITC-dextran (data not shown).

Treatment with SP-A or C1q affects DC chemotaxis. The ability of DCs to stimulate T cells in lymphoid tissues is dependent on the regulated expression of chemokine receptors (55). Immature DCs are recruited to sites of inflammation in peripheral tissues by chemokines that signal through CCR1, CCR2, or CCR5 chemokine receptors. On maturation, these chemokine receptors are downregulated on DCs, with the induction of the CCR7 receptor and a regulated migration of mature DCs into lymph nodes. To assess whether the stimulation of immature BMDCs by SP-A or C1q results in an altered migratory capacity, we tested the chemotaxis of BMDCs toward the known chemokines RANTES (CCR5) or SLC (CCR7). Treatment of immature BMDCs with SP-A or C1q enhanced DC chemotaxis toward RANTES, although BMDCs pretreated with SP-A or C1q were more migratory in general compared with untreated cells (Fig. 6A). As expected, the migration of BMDCs toward RANTES was decreased by incubation of DCs with LPS, since its receptor, CCR5, is downregulated on mature DCs (55). However, there was a statistically significant increase in the LPS-induced chemotaxis when SP-A was included with LPS in the incubation. In contrast, treatment of immature BMDCs with LPS greatly increased DC chemotaxis toward SLC compared with the untreated condition (Fig. 6B). BMDCs treated with SP-A or C1q showed a decreased chemotaxis toward SLC compared with the untreated or LPS condition.

**DISCUSSION**

This study demonstrates that the collectin SP-A and the structurally homologous C1q interact with immature DCs to negatively regulate DC differentiation.
Treatment of immature DCs with SP-A or C1q, but not SP-D, inhibits the expression of MHC class II and CD86. The allostimulatory, endocytic, and chemotactic functions associated with DC differentiation are also impaired by incubation of DCs with SP-A or C1q. Because DCs are the most potent of APCs to initiate adaptive immune responses, our results suggest that the innate immune molecules SP-A and C1q indirectly modify adaptive immune response by regulating DC functions.

The data presented here support the hypothesis that SP-A inhibits the differentiation of immature DCs into mature DCs; SP-A specifically inhibits the expression of MHC class II and CD86 on BMDCs, with lesser or no effect on the expression of CD11c, CD80, CD40, CD54, or MHC class I. The magnitude and specificity of inhibition by SP-A are consistent with other studies demonstrating inhibitory effects of apoptotic cells (49) or cAMP-elevating agents (29) on murine BMDC differentiation or IL-10 on the phenotypic maturation of both human- (9) and murine-derived DCs (50). However, since mouse BMDCs are inherently heterogeneous (40), we cannot exclude the possibility that either SP-A or C1q may also be affecting the differentiation of immature BMDCs into adherent macrophages or may be indirectly regulating DC differentiation through effects on macrophages.

The effects of SP-A and C1q on DC regulatory molecule expression correlate with an inhibition of various functions associated with DC differentiation. Although SP-A has been shown to directly inhibit T cell proliferation (5), these studies are the first to show that SP-A and C1q indirectly affect T cell activation through interactions with DCs. Likewise, the data show that SP-A and C1q affect the migratory capacity of DCs. Interestingly, the results indicate that SP-A and C1q significantly enhance the basal migration of DCs, since treatment of DCs with SP-A or C1q enhanced migration of BMDCs to buffer alone. However, the stimulation of migration to RANTES is most likely dependent on the chemokine gradient, rather than on an indirect stimulatory effect of the defense collagen on DC migration, since BMDCs pretreated with SP-A and C1q showed decreased migration toward the chemokine SLC. Finally, the enhancement of dextran uptake suggests that SP-A and C1q modulate not only the expression of DC regulatory molecules but also innate immune molecules, such as the MR, that play important roles in pathogen uptake by immature DCs.

The ability of SP-A to regulate the cell surface expression of molecules on immune cells has been previously suggested by studies that show SP-A acts as an activation ligand (reviewed in Ref. 59). Gaynor and colleagues (20) showed that pretreatment of macrophages with SP-A stimulated the phagocytosis of Mycobacterium tuberculosis, which was blocked by the addition of mannan or an anti-MR antibody. Studies by Kabha et al. (26) also showed that enhanced binding of a strain of Klebsiella pneumoniae by macrophages pretreated with SP-A was inhibited by mannan, suggesting that binding was mediated by the MR on macrophages. Likewise, pretreatment of monocytes or macrophages with SP-A resulted in enhanced phagocytosis of IgG- or C4b-coated sheep erythrocytes, presumably due to a functional upregulation of the FcR and CRI receptors by SP-A (51). These studies and the present study suggest that SP-A may play an important role in modulating immune responses by directly regulating the cell surface expression of immune molecules.

The differential regulation of CD80 and CD86 expression on BMDCs by SP-A and C1q was somewhat surprising. However, it has been reported that CD80 and CD86 are differentially regulated by a variety of stimuli including IL-10 (9) and IFN-γ (10) and by exposure to apoptotic cells (49) or the intracellular parasite Toxoplasma gondii (17). Although both co-stimulatory molecules are important and sufficient to enhance T cell activation, it has been suggested that CD86 is the predominant ligand responsible for T cell activation by DCs (25). It is also possible that CD80 and CD86 may play distinct roles in immune regulation. For example, CD80 and CD86 can differentially regulate Th1 vs. Th2 immune responses (reviewed in Ref. 22). Furthermore, it has been proposed that CD80 and CD86 may differentially interact with their cognate receptors on T cells; interaction of CD86 with CD28 promotes costimulation, whereas CD80 may be the primary ligand for CTLA4 to promote negative signaling and tolerance (reviewed in Ref. 22). Thus the differential downregulation of CD86 by SP-A and C1q may be an important mechanism for the control of T cell responses.

As expected by their structural similarity, SP-A and C1q share some, but not all, of the same functions. Both SP-A and C1q play a role in the innate immune response to enhance the uptake of microorganisms (52), apoptotic cells (42), and opsonized particles (51) by immune cells. However, SP-A is unable to substitute for C1q to activate complement (57). Some of the similarities in their function may be due to a shared receptor, C1qRp, which plays a role in the SP-A- and C1q-mediated enhancement of phagocytosis (41), although not all functions of C1q are mediated through this receptor (21). Recently, C1qRp was discovered to be homologous to CD93 and expressed on immature but not mature DCs (18, 48). It is intriguing to hypothesize that C1qRp may be the receptor responsible for the inhibition of DC maturation reported in the present study. However, the fact that SP-A and C1q showed similar, yet distinct, effects on DC differentiation suggests that these proteins may not be acting via a shared receptor on DCs. Likewise, the binding of SP-A to BMDCs is not different between immature and mature DCs (unpublished observations), although it is likely that multiple receptors may exist for SP-A on immune cells including DCs (reviewed in Ref. 59). Further studies are necessary to provide insight into the domains and receptors responsible for the observed effects of SP-A and C1q on DC differentiation.

There are several potential mechanisms that may account for the inhibitory effects of SP-A and C1q on
DC functions. First, SP-A and C1q may directly stimulate the production of inhibitory mediators by DCs, such as IL-10 or TGF-β, which have been shown to have similar suppressive effects on DC functions as the defense collagens, such as directly inhibiting the expression of MHC class II and CD86 on DCs, inhibiting DC-induced T cell proliferation, and affecting DC endocytosis and chemotaxis (9, 32, 46, 50). Second, it is possible that SP-A and C1q may regulate the expression of the IL-10 receptor (IL-10R1/R2) on immature DCs and thus modulate the ability of IL-10 to affect DC functions without directly regulating the production of IL-10. A recent study supports this possibility, since the cell surface expression of the IL-10R1 subunit is reduced on mature DCs compared with immature DCs (13). Finally, the defense collagens may initiate signaling events that are directly responsible for the suppressive effects on DC function; the inhibition of DC function by IL-10 has been linked to STAT3 and STAT1 tyrosine phosphorylation (13), whereas TNF-α-mediated activation of DCs induces tyrosine phosphorylation of specific mitogen-activated protein kinases (MAPKs) including ERK2, SAPK/JNK, and p38\textsuperscript{mapk} (46). This latter mechanism is intriguing since SP-A has previously been reported to initiate tyrosine phosphorylation events in macrophages (47). The elucidation of the mechanism by which SP-A and C1q inhibit DC differentiation is an important area of future study.

The modulation of DC function by SP-A and C1q reported in these studies supports a role for collectin-like proteins in linking the innate and adaptive immune response, a prospect that is gaining increased interest (60). Several studies have shown that collectins inhibit mitogen-induced T cell proliferation (6, 7, 56) and more recently that this inhibition of T cell proliferation is due to a direct effect of SP-A and SP-D on CD4\(^+\) T cells (5). We have also recently demonstrated a role for the collectins in enhancing pathogen uptake by DCs, although SP-D was the only protein to also enhance antigen presentation to antigen-specific T cells in this study (8). Together with the present study, these data support a model by which defense collagens interact with DCs in peripheral tissues to differentially modulate the initiation of T cell responses on migration of the DCs to lymphoid organs. Whereas C1q and SP-A inhibit DC differentiation and T cell activation, SP-D enhances DC phagocytosis and would thereby promote the initiation of immune responses to bacterial pathogens in regional lymph nodes. Defense collagens also act locally in the tissues to control T cell responses, thereby minimizing damage to the tissue caused by inflammatory products. This model is most likely an oversimplification, and we postulate that defense collagens may play diverse roles in regulating adaptive immune responses, depending on the pathogen or immune status, as has been described for the regulation of innate immune responses by SP-A (60). Likewise, on the basis of recent findings from SP-A-deficient mice after challenge with influenza A virus, we hypothesize that SP-A may modulate Th1 vs. Th2 immune responses through interactions with DCs (34). The idea that innate immune molecules are critically important in determining the course of an immune response provides a strong rationale for future studies to elucidate the mechanisms by which collectins and the homologous protein C1q regulate adaptive immune responses.

In summary, these data identify a new role for SP-A and C1q in limiting T cell responses in the adaptive immune response. Because DCs are the most potent APC responsible for activating naive T cells, the regulation of DC differentiation is an important mechanism for controlling the initiation of immune responses. Understanding how SP-A and C1q, but not SP-D, interact with purified DC populations to modulate their functional maturation will provide insight into the differential regulation of immune cell functions by soluble innate immune molecules. Importantly, continued efforts toward understanding the role of defense collagens in regulating adaptive immune responses may provide a basis for using collectins or C1q as therapeutic agents against damaging immune diseases.

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REFERENCES

Surfactant protein A (SP-A) regulates DC differentiation


