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# A Filarial Nematode-Secreted Product Signals Dendritic Cells to Acquire a Phenotype That Drives Development of Th2 Cells<sup>1</sup>

Michael Whelan,\* Margaret M. Harnett,<sup>†</sup> Katrina M. Houston,<sup>‡</sup> Vanshree Patel,\* William Harnett,<sup>‡</sup> and Kevin P. Rigley<sup>2\*</sup>

Although exogenous “danger” signals such as LPS can activate APC to produce a Th1 response, the nature of events initiating a Th2 response is controversial. We now show that pathogen-derived products have the capacity to induce bone marrow-derived dendritic cell cultures to acquire a phenotype that promotes the differentiation of naive CD4<sup>+</sup> T cells toward either a Th1 or Th2 phenotype. Thus, LPS-matured dendritic cells (DC1) promote a Th1 response (increased generation of IFN- $\gamma$  and reduced production of IL-4) by Ag-stimulated CD4<sup>+</sup> T cells from the DO.11.10 transgenic mouse expressing a TCR specific for an OVA peptide (OVA323–339). In contrast, a phosphorylcholine-containing glycoprotein, ES-62, secreted by the filarial nematode, *Acanthocheilonema viteae*, which generates a Th2 Ab response in vivo, is found to induce the maturation of dendritic cells (DC2) with the capacity to induce Th2 responses (increased IL-4 and decreased IFN- $\gamma$ ). In addition, we show that the switch to either Th1 or Th2 responses is not effected by differential regulation through CD80 or CD86 and that a Th2 response is achieved in the presence of IL-12. *The Journal of Immunology*, 2000, 164: 6453–6460.

The adaptive immune response plays a critical role in the eradication of pathogens. However, inappropriate responses to infection can cause severe pathology. Thus, for effective vaccine design it is of crucial importance to understand the underlying processes that occur as a result of the interaction of pathogens with the innate immune system and, in particular, how such interactions lead to particular categories of the adaptive immune response. Naive T cells differentiate into discrete subsets of cytokine-secreting cells such as those represented by the Th1 and Th2 phenotypes of Th cells (1, 2). However, the nature of the early differentiation signals leading to this phenotype commitment is poorly understood. While it is clear that exogenous “danger” signals such as LPS activate APC to promote a Th1 response, the nature of the cell types or molecules involved in initiating a Th2 response has yet to be clearly described (3). Dendritic cells (DC)<sup>3</sup> are specialized APCs required for the priming and activation of CD4<sup>+</sup> T cells and, as such, could potentially direct the subsequent differentiation of T cell function (4). However, there is a widely held view that whereas exogenous pathogen-derived products induce DC maturation to promote the default differentiation of CD4<sup>+</sup> T cells toward a Th1 phenotype, B cells are required to provide the signals to CD4<sup>+</sup> T cells that result in the promotion of Th2 cell development (5). This view has been supported by the

lack of evidence, to date, for the ability of pathogen-activated DC to promote a Th2 response. However, the recent finding that DC derived from myeloid and lymphoid lineages can promote Th1 and Th2 responses, respectively (6), supports the alternative view that differential maturation might also induce DCs to acquire the ability to promote a Th2 response. Therefore, we initiated the present study to test the hypothesis that the information required to elicit the qualitative nature of the adaptive immune response is 1) found within the pathogen and 2) decoded by cells of the innate immune system. This simple idea provides a rationale for explaining the outcome of an immune interaction and would be consistent with recent data indicating that commitment to a particular class of T cell response occurs early during immune responsiveness (7). To explore this hypothesis, we used a well-characterized in vitro Th cell assay in which CD4<sup>+</sup> T cells from the DO.11.10 transgenic (Tg) mouse express a TCR that is specific for the OVA peptide (323–339) (8). When these naive CD4<sup>+</sup> T cells are cultured with bone marrow-derived DC (bm-DC) in the presence of OVA peptide, they secrete both IFN- $\gamma$  and IL-4. This reflects the capacity of these cells to generate either a Th1 or a Th2 response (9) and thus provides a simple model of immune modulation.

To determine whether bm-DC can be matured to bias CD4<sup>+</sup> T cells to either a Th1 or Th2 phenotype, we embarked on a screening program in which immature DC were precultured with a variety of pathogen products and then used to stimulate cytokine production in such OVA-specific CD4<sup>+</sup> cells. While LPS was used to mature DC (DC1) to promote a Th1 response, a phosphorylcholine-containing glycoprotein, ES-62, secreted by the filarial nematode, *Acanthocheilonema viteae*, was found to induce the maturation of DC (DC2) with the capacity to induce Th2 responses. This pathogen product was chosen because filarial nematodes are parasites that have a propensity to generate a Th2 response in vivo, and such Th2 responses are considered to be associated with parasite longevity (10). Moreover, we had previously shown that this phosphorylcholine-containing glycoprotein, ES-62, has the capacity to modulate lymphocyte activation in vitro and in vivo (11). Thus, we now provide the first evidence that

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<sup>3</sup> Abbreviations used in this paper: DC, dendritic cell; Tg, transgenic; bm-DC, bone marrow-derived DC.

immature bm-DC can be activated in the presence of pathogen products to generate the signals necessary for either a Th1 or Th2 response. These results suggest that DCs in the innate immune system can act as a cellular differentiation “bridge” between pathogens and the subsequent adaptive response.

## Materials and Methods

### Animals

BALB/c mice were bred at the Institute for Animal Health (Compton, Berkshire, U.K.) and the Department of Immunology, University of Strathclyde (Glasgow, U.K.). A DO.11.10  $\alpha\beta$  TCR Tg mouse founder colony was a kind gift from Dr. Fiona Powrie (Nuffield Department of Surgery, Oxford, U.K.). All Tg mice were assayed for the transgene using a biotinylated KJ1-26 mAb (a gift from Dr. Powrie) to stain splenocytes for flow cytometric analysis. All mice used were 6–12 wk old. The life cycle of the rodent filarial nematode, *A. viteae*, was maintained at the University of Strathclyde as described previously (12).

### Preparation of the phosphorylcholine-containing glycoprotein, ES-62

ES-62 was purified from spent culture medium of adult *A. viteae* by ultrafiltration as described previously (12).

### Cell culture

Routine tissue culture of splenocytes and sorted T cells was conducted in RPMI 1640 Glutamax I (Life Technologies, Paisley, U.K.), supplemented with 10% heat-inactivated FCS (Life Technologies), 50  $\mu\text{M}$  2-ME (Sigma, Poole, Dorset, U.K.), 100 U/ml penicillin, and 100  $\mu\text{g}/\text{ml}$  streptomycin (Life Technologies). Bm-DC isolation medium consisted of MEM with Earle's salts, 25 mM HEPES, and Glutamax I (Life Technologies) supplemented with 2% FCS, while bm-DC culture medium comprised IMDM with Glutamax I, 25 mM HEPES (Life Technologies) supplemented with 10% FCS, 50  $\mu\text{M}$  2-ME, and 10 ng/ml GM-CSF (R&D Systems, Abingdon, Oxon, U.K.).

### Flow cytometry

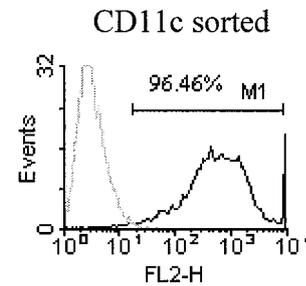
FITC-conjugated Abs specific for CD4, CD8, B7.1 (CD80), B7.2 (CD86), CD54, and CD40 were purchased from Harlan SeraLabs (Loughborough, Leicestershire, U.K.) along with the relevant isotype controls. MHC class II expression was measured using a FITC-conjugated anti-I-A<sup>d</sup>/I-E<sup>d</sup> Ab (PharMingen, Oxford, U.K.). Flow cytometry was conducted using a FACScalibur Immunocytometry System (Becton Dickinson, Oxford, Oxon, U.K.).

### Monoclonal Abs

Blocking mAb to IL-12 (C17.8), IL-10 (JES5-2A5), CD80 (1G10), and CD86 (PO3) were all purchased from PharMingen. CTLA-4-Ig was obtained from R&D Systems.

### Culture of bm-DC and treatment with modulins

Femurs and tibias were dissected from mice as previously described (13) and bone marrow cells seeded into 75-cm<sup>2</sup> flasks containing bm-DC culture medium at 37°C in a humidified incubator. On day 4, the supernatant was carefully removed from the flasks and the cells replenished with fresh bm-DC culture medium. On day 6, loosely adherent cells were removed by gentle pipetting and centrifugation and used as immature bm-DC. Such bm-DC show a marked degree of heterogeneity for many surface molecules although they are routinely negative for F4/80 (macrophage), CD19 (B cell), CD3 (T cell), and Gr-1 (granulocytes) (results not shown). In some experiments presented, these cultures were used as a source of DC. In other studies, bm-DC were further purified to >95% purity using magnetic anti-CD11c beads (Fig. 1) following the protocol provided the manufacturer (Miltenyi Biotec, Surrey, U.K.). Following isolation, bm-DC or, where indicated, CD11c<sup>+</sup> bm-DC were plated at  $1 \times 10^6$  cells/ml in 24-well plates (Falcon; Becton Dickinson) and further grown in bm-DC culture medium in the presence or absence of LPS (*Escherichia coli* serotype 055: B5, Sigma) (1–10  $\mu\text{g}/\text{ml}$ ) or ES-62 (1–2  $\mu\text{g}/\text{ml}$ ) for a further 24 h at 37°C. The cells were then washed three times with PBS before coculturing with naive CD4<sup>+</sup> DO.11.10 Tg T cells. Essentially the same results were obtained with bm-DC or CD11c plus bm-DC.



**FIGURE 1.** Isolation of CD11c plus bm-DC. Bm-DC were cultured as described in *Materials and Methods*. At day 6, CD11c plus DC were isolated by magnetic cell sorting. The resulting bm-DC population was found to be >95% CD11c<sup>+</sup> (solid line).

### Purification of CD4<sup>+</sup> CD62L<sup>high</sup> T cells and DNA synthesis assays

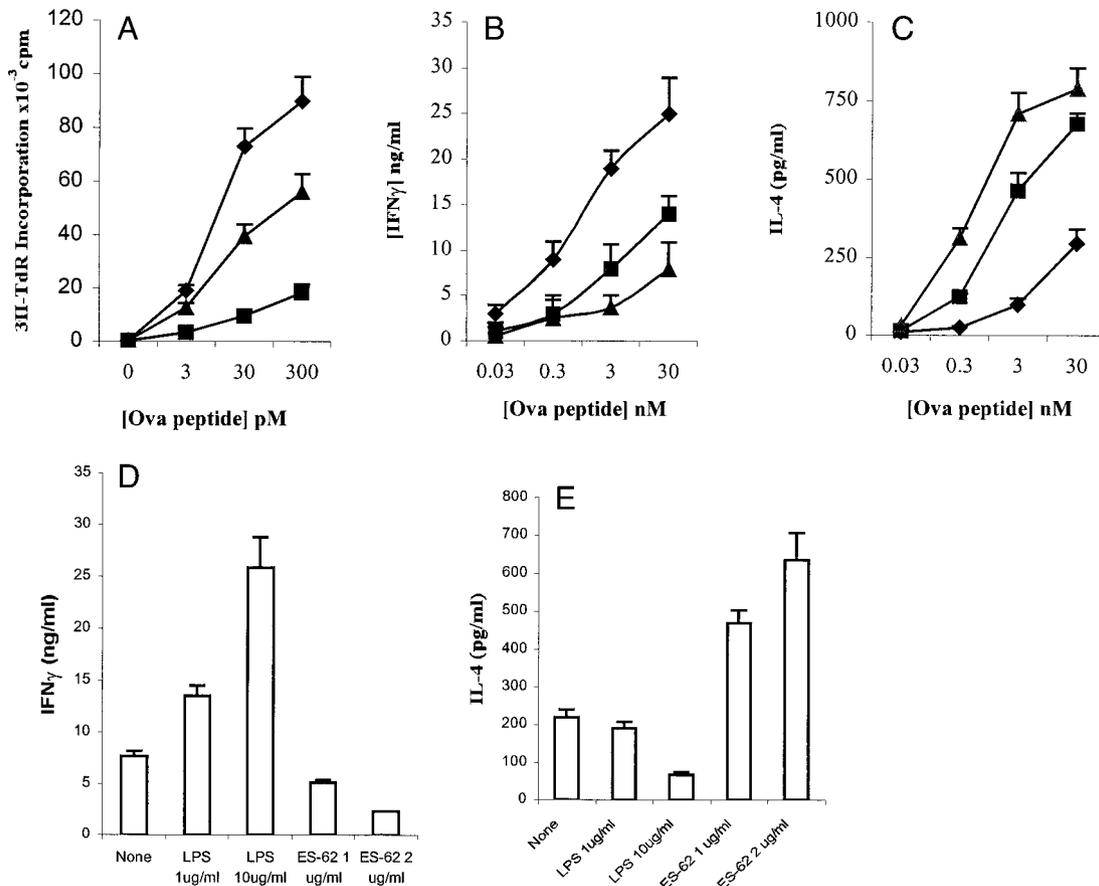
Purified naive CD4<sup>+</sup> CD62L<sup>high</sup> T cells from DO.11.10 mice were used as source of responder T cells for bm-DC derived from BALB/c mice. Splens were removed from DO.11.10 mice and passed through 70- $\mu\text{m}$  cell strainers (Falcon; Becton Dickinson). The splenocytes were then washed in RPMI 1640 and resuspended ( $10^8$  cells/ml) in magnetic activated cell sorter (MACS) buffer (FACSFlow; Becton Dickinson; 0.5% FCS) and incubated with anti-CD4-MACS beads (Miltenyi Biotec). Following purification, the CD4<sup>+</sup> cells were subsequently stained with anti-CD62L-FITC and anti-CD4-PE (PharMingen) to allow purification of the naive cells (CD62L<sup>high</sup>CD4<sup>+</sup>) by FACS sorting. Purified naive (CD62L<sup>high</sup>CD4<sup>+</sup>) Tg T cells ( $10^5$  cells/well) were then cultured in RPMI 1640 plus 10% FCS with the relevant DC and various concentrations of the OVA peptide (323-ISQAVHAAHAEINEAGR-339; obtained from Genosys Europe, Pampisford, Cambridgeshire, U.K.) for 3 days at 37°C. Cells were then pulsed overnight with [<sup>3</sup>H]thymidine (1  $\mu\text{Ci}/\text{well}$ ; Amersham, Bucks, U.K.), and incorporation into DNA was analyzed following cell harvesting (Tomtec cell harvester; Wallac, Turku, Finland) by liquid scintillation counting (MicroBeta scintillation counter; Wallac).

### Cytokine assays

CD4<sup>+</sup> T cells were isolated as described above and cultured ( $2.5 \times 10^5$  cells) with either irradiated (2500 rad;  $\gamma$  source) bm-DC cultures or CD11c-purified bm-DC (as indicated) ( $2.5 \times 10^4$  cells) in a total volume of 1 ml in 24-well tissue culture plates (Nunc, Naperville, IL) with or without OVA peptide at various concentrations for 3 days at 37°C. The cells were then stimulated with 50 ng/ml PMA (Sigma) and 500 ng/ml ionomycin (Sigma) overnight. The cell supernatants were then removed and tested for expression of IL-2, IL-4, IFN- $\gamma$ , IL-10, and IL-12 p70 by ELISA (R&D Systems).

### Measurement of IgG1/IgG2a by ELISA

Flat-bottom 96-well plates were coated with PBS, pH 9, containing 1  $\mu\text{g}/\text{ml}$  of ES-62 (100  $\mu\text{l}/\text{well}$ ) and incubated overnight at 4°C. After washing three times with PBS/Tween (0.05% Tween 20 in PBS, pH 7.4), 150  $\mu\text{l}$  of 4% BSA in PBS was added to each well, and plates were incubated for 1 h at 37°C. The plates were then washed as above, and 100  $\mu\text{l}$  of serum serially diluted 1:3 (starting at 1:100) in PBS/Tween was added to duplicate wells and incubated for 1 h at 37°C. After washing, 100  $\mu\text{l}$  of HRP-conjugated Abs specific for IgG1 or IgG2a, diluted 1:20,000 or 1:10,000, respectively, in PBS containing 25% (v/v) sheep serum, were added to each well. Following a 1-h incubation at 37°C and a subsequent wash, 100  $\mu\text{l}$  of substrate solution was added to each well. This solution was prepared by adding 8  $\mu\text{l}$  of hydrogen peroxide and 250  $\mu\text{l}$  of 6 mg/ml tetramethylbenzidine to 25 ml of 0.1 M sodium acetate solution, pH 5.5. The enzymatic reaction was allowed to proceed for 15 min in darkness at room temperature before being stopped by the addition of 50  $\mu\text{l}$  of 10% (v/v) sulfuric acid. The absorbencies were then read at 450 nm on a Titertek Multiskan. Results were expressed as reciprocal endpoint dilutions.



**FIGURE 2.** LPS and ES-62 promote the differentiation of bm-DC toward either a DC1 or DC2 phenotype. Bm-DC matured by GM-CSF in the presence of 1  $\mu$ g/ml LPS (◆), 1  $\mu$ g/ml ES-62 (▲), or GM-CSF (■) were washed and then cocultured with DO.11.10 Tg CD4<sup>+</sup> T cells at the indicated concentrations of OVA peptide before measuring DNA synthesis (a), IFN- $\gamma$  production (b), or IL-4 production (c). Data are presented as means  $\pm$  SD from single experiments representative of at least three other independent experiments. In d and e, data pooled from an additional five independent experiments using CD11c<sup>+</sup> bm-DC (means  $\pm$  SD) are presented showing that while maturing DC in the presence of increasing concentrations of LPS acts to further promote IFN- $\gamma$  and suppress IL-4 production, maturation in the presence of increasing concentrations of ES-62 results in the more pronounced abrogation of IFN- $\gamma$  and enhanced production of IL-4 from OVA (3 nM)-stimulated DO11.10 Tg CD4<sup>+</sup> T cells.

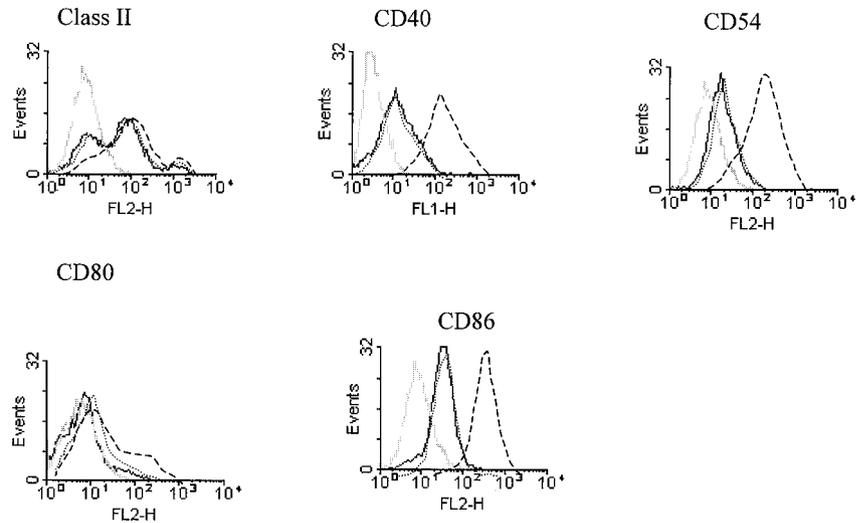
## Results

### *LPS and ES-62 promote the differentiation of bm-DC toward either a DC1 or DC2 phenotype*

It has been shown previously that murine bm-DC pulsed with varying doses of peptide (14) and human CD14<sup>+</sup> DC cultured in the presence of PGE<sub>2</sub> (15) can promote differential regulation of T cell cytokine profiles. Moreover, it has also been proposed that pattern recognition receptors expressed on APC can interact with products of pathogens (modulins) to subsequently modify T cell function. To test this idea, we generated DC from mouse bone marrow, exposed these cultures to products of pathogens, and then assessed their ability to modify T cell function in a peptide-specific Tg system. Culture of GM-CSF-matured bm-DC with either LPS (DC-LPS) or ES-62 (DC-ES-62) revealed a DC phenotype that promoted increased proliferation (as indicated by DNA synthesis (Fig. 2a)) of naive CD4<sup>+</sup>CD62L<sup>high</sup> (99% pure, data not shown) Ag-specific Tg T cells at all concentrations of OVA peptide tested (1–300 pM), compared with that observed with DC matured with GM-CSF alone (DC-GM-CSF) (Fig. 2a). Similar enhanced T cell proliferative responses were obtained using either CD11c plus bm-DC as stimulators or when alloreactive responder T cells were used (data not shown). To determine whether the observed increase in proliferation was associated with either Th1 or Th2 cytokine production, we cocultured either bm-DC or CD11c<sup>+</sup>

bm-DC matured under all three conditions together with OVA-specific T cells and measured the production of the signature Th1 cytokine, IFN- $\gamma$ , and the canonical Th2 cytokine, IL-4. The data shown (Fig. 2b) is derived from experiments using bm-DC although essentially identical results were obtained using CD11c with or without DC (results not shown). We found (Fig. 2b), as might have been predicted from earlier studies showing that LPS-treated APC promote a Th1 profile of cytokine secretion in CD4<sup>+</sup> T cells that compared with DC-GM-CSF (DC0), DC-LPS do indeed promote an increase in IFN- $\gamma$  (16, 17). In contrast, DC-ES-62 did not mediate such an increase in IFN- $\gamma$  but rather, at concentrations of OVA peptide >1 nM, appeared to inhibit IFN- $\gamma$  production to below the levels obtained following culture with DC0 and Tg T cells (Fig. 2b). As it has been widely established that IL-4 can suppress IFN- $\gamma$  production (18), we speculated that the diminished IFN- $\gamma$  production observed in DC-ES-62/CD4<sup>+</sup> T cell cocultures might be due to the preferential outgrowth of IL-4-producing Th2 cells. As can be seen from the results presented in Fig. 2c, this is indeed the case: while DC-LPS suppresses IL-4 production, DC-ES-62 promotes a significant increase in IL-4 levels compared with those observed in response to DC-GM-CSF. As it has been shown previously that high Ag doses generally favor a Th1 response (14), we investigated whether these differential pathogen-mediated effects on DC maturation simply reflected distinct

**FIGURE 3.** LPS and ES-62 exhibit differential effects on expression of costimulatory molecules on CD11c plus bm-DC. CD11c<sup>+</sup> bm-DC were matured with GM-CSF either alone or in combination with LPS (1  $\mu\text{g}/\text{ml}$ ) or ES-62 (2  $\mu\text{g}/\text{ml}$ ). Cell-surface Ag expression was analyzed by flow cytometry as described in *Materials and Methods*. Data are presented as histograms from a single experiment representative of several others and is presented as fluorescent intensity (FL1/FL2) of Ag staining vs cell number. Gray broken line, negative control Ab staining; black line, staining of DC-GM-CSF; black broken line, staining of DC-LPS cultures; black dotted line, staining of DC-ES-62 cultures. The data presented are from a single experiment representative of three other independent experiments.



Ag dose thresholds for these pathogen products. However, data from five independent experiments using CD11c<sup>+</sup> DC with or without bm-DC clearly showed that while increasing the concentration of LPS (from 1 to 10  $\mu\text{g}/\text{ml}$ ) does indeed further polarize the resulting T cell response toward a Th1-like phenotype (enhances IFN- $\gamma$  (Fig. 2*d*) and almost completely suppresses IL-4 production (Fig. 2*e*), increasing the concentration of ES-62 (from 1–2  $\mu\text{g}/\text{ml}$ ) to which the DC are exposed further biased the response toward a Th2 phenotype (IFN- $\gamma$  production is almost completely suppressed (Fig. 2*d*) and IL-4 generation is further enhanced (Fig. 2*e*)).

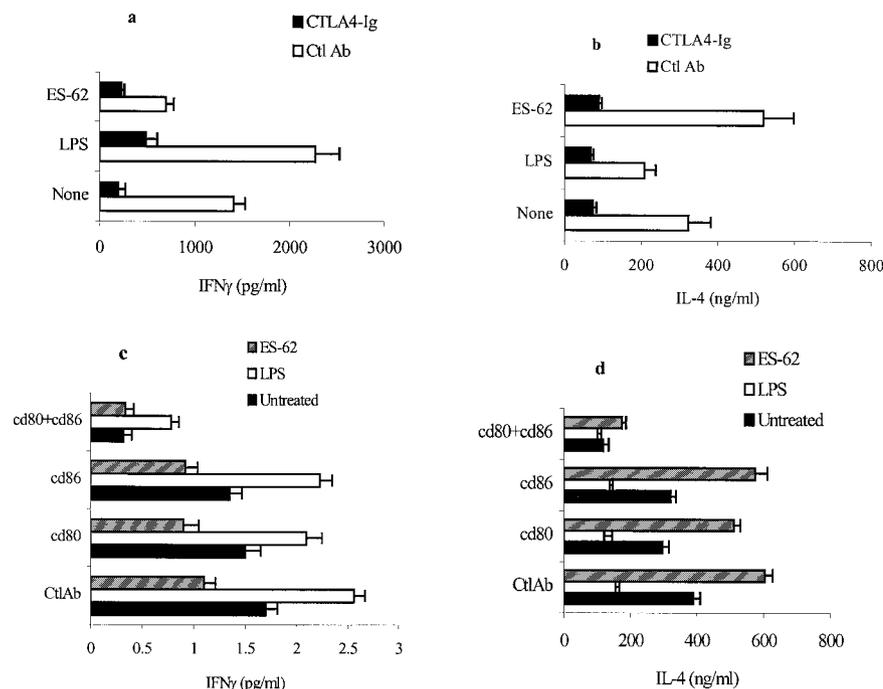
#### Differential effects of LPS and ES-62 on expression of costimulatory molecules during bm-DC maturation

DC express on their cell surface a variety of molecules that are known to be important for both T cell activation and differentiation (4). However, no single molecule has been identified as being responsible for driving the differentiation of Th0 cells toward Th1 or Th2 phenotypes. To further explore the mechanism by which DC can induce differential T cell cytokine profiles, we used CD11c<sup>+</sup>

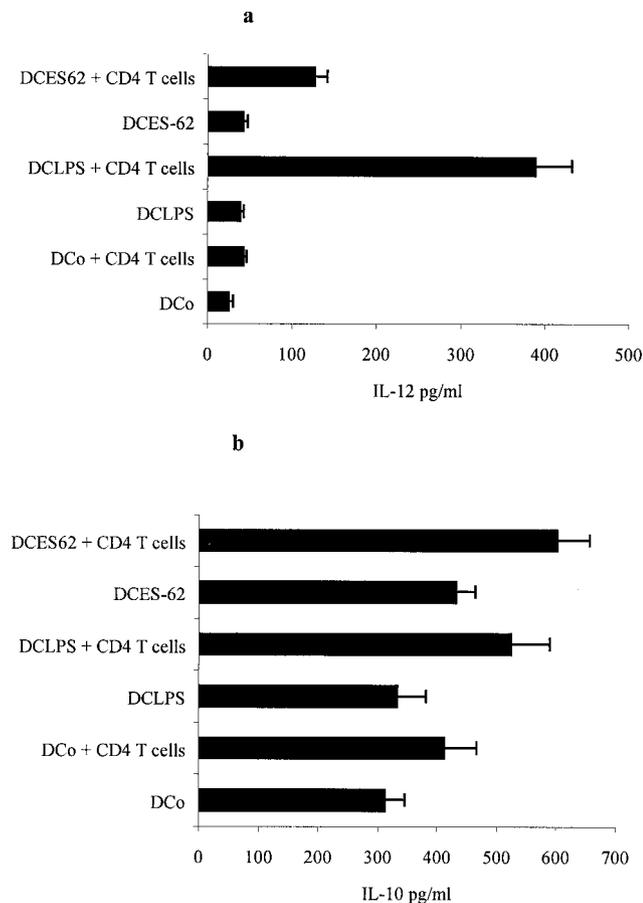
DC to look for differential expression of candidate molecules by either DC-LPS (DC1) or DC-ES-62 (DC2) (Fig. 3). These data clearly show that DC-LPS demonstrate increased expression of CD40, B7.1 (CD80), B7.2 (CD86), and CD54 relative to DC-GM-CSF. In contrast, DC-ES-62 do not show an increased expression of any of these markers relative to DC-GM-CSF. Similar experiments with unpurified bm-DC basically provided identical results (results not shown). Consistent with our findings above (Fig. 2, *d* and *e*) that increasing the Ag dose does not necessarily favor development of a Th1 response, we found that MHC class II expression did not change substantially in either route of DC differentiation from the, albeit high, levels of MHC class II expression observed in DC-GM-CSF cells.

#### CD80 and CD86 do not mediate DC-mediated development of Th phenotype

CD80 and CD86 were up-regulated on bm-DC matured in the presence of LPS but not ES-62 or GM-CSF alone (Fig. 3). Thus,



**FIGURE 4.** Blocking CD80/CD86 and CD28/CTLA4 interactions inhibits DC-LPS- and DC-ES-62-induced cytokine production. The effect of culturing DC-LPS, DC-ES-62, and DC-GM-CSF with control Ig (10  $\mu\text{g}/\text{ml}$ ) or a CTLA4-Ig fusion protein (10  $\mu\text{g}/\text{ml}$ ) (a and b) or blocking Abs to CD80 (10  $\mu\text{g}/\text{ml}$ ; clone IG10; PharMingen) and CD86 (10  $\mu\text{g}/\text{ml}$ ; clone PO3; PharMingen) either alone or together (c and d) on IFN- $\gamma$  (a and c) or IL-4 (b and d) production. Data are presented as means  $\pm$  SD from experiments representative of two other independent experiments



**FIGURE 5.** Induction of IL-12 and IL-10 by DCs matured with modulators. CD11c<sup>+</sup> DC-GM-CSF, DC-LPS, or DC-ES-62 were cultured either alone or in the presence of CD4<sup>+</sup> DO.11.10 T cells and OVA peptide (3 nM) for 48 h. Supernatants were removed and analyzed by ELISA for production of (a) p70 IL-12 protein or (b) IL-10. Data is presented as means  $\pm$  SD from a single experiment representative of two others.

to address whether CD80/CD86 played a key role in the LPS-mediated development of a Th1 phenotype, we repeated our experiments in the presence of a soluble CTLA4-Ig fusion protein (Fig. 4, *a* and *b*) to block bidirectional signaling between CD80/CD86 on the DCs with CD28/CTLA-4 counter-structures on the OVA-specific DO11.10 Tg T cells. These results showed that while culture with CTLA4-Ig did indeed block LPS- and ES-62-mediated IFN- $\gamma$  production, it also blocked ES-62/LPS-mediated IL-4 production. Given that CD80/CD86 have previously been implicated in the development of polarized Th responses, these results were rather surprising. Thus, to further directly assess the role of CD80 and/or CD86 in DC-directed Th1/Th2 differentiation, we also tested the effect of blocking mAbs to these molecules, either alone or in combination, on the OVA-specific production of either IFN- $\gamma$  or IL-4 (Fig. 4, *c* and *d*). Our data show that while both Th1 and Th2 cytokine production is only partially blocked with either Ab alone, almost complete inhibition of both types of cytokine production is seen when blocking with anti-CD80 and anti-CD86 Abs together (Fig. 4, *c* and *d*).

#### Reciprocal regulation of DC1 and DC2 by IL-10 and IL-12

DC have been reported to produce a number of cytokines that are known to influence T cell differentiation (19). In particular, IL-12 and IL-10 have been proposed to reciprocally influence the induction of IFN- $\gamma$  by Th1 cells and Th2 cells respectively (19). To

address the role of these cytokines in our system, we measured production of either IL-12 or IL-10 by all three CD11c<sup>+</sup> DC phenotypes (Fig. 5, *a* and *b*); no significant production of p70 IL-12 was detected following culture of DC-GM-CSF, DC-LPS, or DC-ES-62 alone. However, following coincubation with OVA-specific CD4<sup>+</sup> T cells, p70 IL-12 production was dramatically up-regulated in DC-LPS/T cell cultures (Fig. 5*a*). Unexpectedly, a smaller but significant increase in p70 IL-12 production was also observed in DC-ES-62 but not DC-GM-CSF/T cell cocultures (Fig. 5*a*).

IL-10 was found to be produced by all phenotypes of CD11c<sup>+</sup> bm-DCs including DC-LPS cultures, and this production was enhanced by the presence of T cells (Fig. 5*b*). As there was no significant difference in the levels of IL-10 produced by cocultures containing DC-ES-62 or DC-LPS, these results suggested that IL-10 might not be responsible for the switch from DC0 to DC2 and consequent IL-4 production. However, these results did not preclude the possibility that any differential IL-10 might be receptor bound or used up at different rates, events that would influence the levels of available free IL-10 for detection. Thus to further address the role of these cytokines in our system, we tested the effects of either recombinant IL-12, IL-10, or their respective appropriate neutralizing Abs on cytokine production by our CD11c<sup>+</sup> bm-DC/T cell cocultures (Fig. 6).

Perhaps unsurprisingly, we found that addition of IL-12 promotes an increase in IFN- $\gamma$  production by all phenotypes of CD11c<sup>+</sup> bm-DC/T cell cocultures (Fig. 6*a*). Similarly, incorporation of neutralizing anti-IL-12 mAb diminished IFN- $\gamma$  production in all the cultures, thereby confirming the key role of this cytokine in promoting IFN- $\gamma$  production (8, 20). In contrast, although addition of anti-IL-12 was found, as expected, to substantially increase IL-4 in these same culture supernatants, addition of rIL-12 only slightly suppressed IL-4 levels, consistent with our previous findings that DC-ES-62 promoted a Th2 phenotype despite the production of substantial levels of p70 IL-12 (Fig. 6*b*).

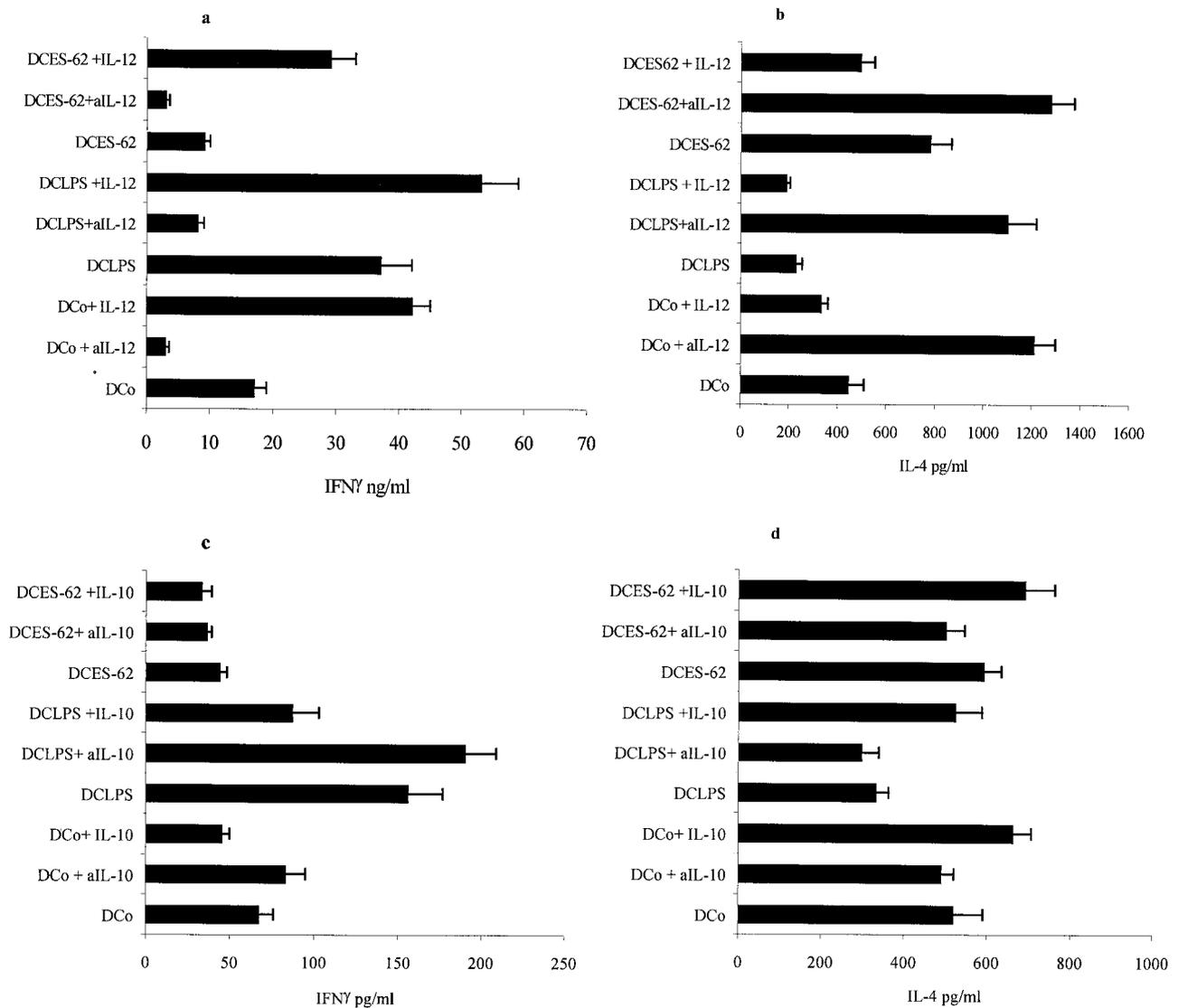
The effects of modulating IL-10 levels in these CD11 plus bm-DC/T cultures was less clear cut; although anti-IL-10 slightly promoted whereas rIL-10 slightly suppressed IFN- $\gamma$  production by DC-LPS/T cell cultures, these reagents had only marginal effects on cultures containing either DCo or DC-ES-62 cells (Fig. 6*c*). Moreover, whereas addition of rIL-10 appeared to weakly promote an increase in IL-4 production, anti-IL-10 did not appear to have any significant effect on the secretion of this cytokine by any of the DC/T cell cultures (Fig. 6*d*), a finding that is presumably consistent with our data (Fig. 5) showing that DC-LPs and DC-ES-62 cultures do not produce significantly different levels of IL-10 (Fig. 6*d*).

#### ES-62 induces a Th2 response in vivo

Our results thus far are consistent with the idea that the innate immune system, and in particular DC, can be matured by products of pathogens to acquire the ability to bias an immune response toward either a Th1 or Th2 phenotype. One consequence of a Th2 response in vivo is the production of IgG1 in preference to the Th1-induced Ig isotype, IgG2a (in mice). To assess the efficacy of ES-62 in induction of an in vivo Th2 response, we therefore measured IgG1/IgG2a production following s.c. inoculation with ES-62. The results shown in Fig. 7 clearly demonstrate a dramatic increase in serum levels of ES-62-specific IgG1. In contrast, no significant IgG2a response to ES-62 could be detected.

## Discussion

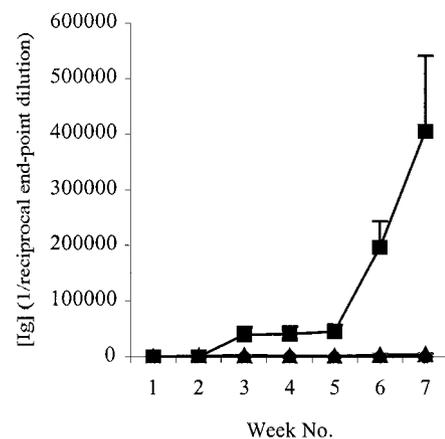
The results presented in this paper clearly indicate that naive immature DC (DC-GM-CSF, designated DCo) can, following exposure to different pathogen products, acquire either a DC phenotype



**FIGURE 6.** The role of IL-12 and IL-10 in modulating Th1 and Th2 differentiation by differentially matured CD11c<sup>+</sup> bm-DC. CD11c<sup>+</sup> DC/T cell cocultures were set up in the presence of OVA (3 nM), and, where indicated, at the time of initiation, cultures additionally received either recombinant IL-12 (25 pg/ml) or neutralizing mAb to IL-12 (10  $\mu$ g/ml) (a and b) or rIL-10 (2ng/ml) or neutralizing mAb to IL-10 (5  $\mu$ g/ml) (c and d). Supernatants were assessed by ELISA for IFN- $\gamma$  (a and c) or IL-4 (b and d) production. Data are presented as means  $\pm$  SD from a single experiment representative of one other.

that drives a Th1 response (DC-LPS, designated DC1) or a Th2 response (DC-ES-62, designated DC2). Therefore, these data are in direct contrast to the widely held view that whereas Ag presentation by DC results in the induction of a Th1 phenotype, B cells are required for the development of a Th2 response (5) and suggest that the signals dictating the phenotype of the adaptive immune response are intrinsic to the pathogen product and can be decoded by cells of the innate immune system.

To address the mechanisms by which such pathogen-matured DC1 and DC2 phenotypes influence Th cell development, we evaluated the role of a variety of costimulatory molecules and cytokines in the regulation of Th1 and Th2 cytokine production. First, as it had previously been proposed that increasing the Ag dose favors development of a Th1 response (14, 21), we investigated whether DC1 and DC2 cells exhibited differential MHC class II expression; however, we found not only that MHC class II expression did not change significantly in either route of DC differentiation (Fig. 3) but also that while increasing the dose of LPS did indeed enhance Th1 development, increasing the dose of ES-62



**FIGURE 7.** ES-62 elicits IgG1 but not IgG2a Ab production in vivo. Weekly serum samples were taken from BALB/c mice given weekly s.c. injections of ES-62 (3  $\mu$ g) and assayed for specific anti-ES-62 IgG1 (■) and IgG2a (▲) Abs by ELISA. Results are expressed as reciprocal endpoint dilution from a single experiment representative of at least two other independent experiments.

promoted further polarization to the Th2 phenotype (Fig. 2). Therefore, these results support our proposal that the subsequent differentiation of T cells toward a Th1 or Th2 phenotype in our system is dependent on the nature of the pathogen product rather than due to an Ag dose effect as has been suggested by others (14, 22).

Why would the immune system default to a Th1 pathway at high Ag doses? Previous studies showing induction of a Th1 phenotype in response to increasing doses of peptide administered *in vivo* (14) might simply reflect selective targeting of certain class II-expressing APC, known to promote development of Th1 cell function (8), which would not necessarily occur during a normal immune response following infection with complex pathogen Ags such as parasites. Alternatively, it has been suggested that signals from certain costimulatory molecules converge on transduction events emanating from the TCR, effecting changes in both duration and amplitude of these signal transduction pathways. Previous findings that Th1 responses can be induced by high Ag concentration may therefore simply reflect enhancement of a particular pattern of increased expression of costimulatory activity and an increase in the relative avidity of Ag-specific T cell/DC conjugates. In this regard, CD80 and CD86 have been implicated in the costimulatory process and in IFN- $\gamma$  production, although their exact role with respect to the latter is controversial (23, 24). Although we found that CD80 and CD86 were expressed at higher levels in DC-LPS than in DC-ES-62, our data using CTLA-4-Ig fusion proteins and the appropriate neutralizing Abs (Fig. 4) suggest that CD80 and CD86 are required for both IL-4 and IFN- $\gamma$  cytokine production, presumably via IL-2 production and T cell expansion, but are not involved in transmitting DC-derived differentiation signals. Thus, although our data differs from earlier reports using CD28- or CD80/86-deficient cells, which suggested that these interactions play a role in Th2 development (25), it is consistent with reports proposing that the predominant contribution of CD80/86 might be to promote proliferation rather than differentiation (24). In contrast, our finding that DC-LPS express higher levels of CD54 than DC-ES-62 might be consistent with recent reports that costimulation via ICAM-1 (CD54) suppresses the induction of IL-10 and favors the development of Th1 cells (26). Furthermore, the combination of low expression of CD54 and CD80 by DC-ES-62 relative to that observed with DC-LPS may reflect reports that low levels of CD80 expression have been observed on PBMCs of individuals infected with filarial nematodes (who will have parasite phosphorylcholine-containing glycoproteins secreted into their bodies) who demonstrate a poor capacity to make IFN- $\gamma$  but an enhanced capacity to make IL-10 (27). Therefore, a reasonable explanation for our data is that the interaction of pathogens with DC induces an increase in a particular pattern of costimulatory molecules and/or cytokines that subsequently regulate Th0 differentiation and/or expansion of effector T cells.

All of the candidate costimulatory molecules investigated were shown to be expressed at lower levels on DC-ES-62 relative to DC-LPS cells (Fig. 3), suggesting that the differentiation and enhanced proliferation of OVA-specific Th2 cells observed in response to DC-ES reflects up-regulation of as yet undefined costimulatory molecules during transition from the DC0 to DC2 phenotype. Alternatively, it was possible that Th2 induction was due to the production and/or suppression of cytokines, such as IL-10 or IL-12, which have previously been implicated in the regulation of differential Th phenotypes, by DC-ES-62. This possibility seemed particularly pertinent in the light of previous reports that phosphorylcholine-containing molecules appear to promote Th2 responses, at least in part, by inducing IL-10 (28). Therefore, we were rather surprised to find not only that coculture of T cells

with DC-ES-62 results in a small but significant production of IL-12 but also that such cultures do not produce significantly more IL-10 than those involving DC-LPS (Fig. 5). However, this can be explained by the target for phosphorylcholine in the previous studies being shown to be B1 cells (28). Unlike B1 cells, DCs may not synthesize IL-10 in response to PC or may synthesize much lower amounts. Our findings were borne out by studies employing addition of rIL-10 or neutralizing anti-IL-10 Abs, which suggested that DC-ES-62 may induce Th2 development by a mechanism independent of IL-10. Moreover, and consistent with the idea that IL-4 is the dominant cytokine when used in combination with IL-12 (18), increased IL-4 production occurs even in the presence of IL-12 and provides a mechanism for overriding the ability of this latter cytokine to promote a Th1 response.

In summary, our data clearly support the view that information contained within pathogens is recognized and decoded by DC and that the innate immune system acts as a conduit through which regulation of the adaptive immune response can occur. We propose that DC1 promote a Th1 response and DC2 promote a Th2 response. At present, we are unable to determine whether DC1 and DC2 are derived from a common precursor (DC0) or are expanded from distinct lineages as reported elsewhere (6). However, we have shown that both of these distinct DC phenotypes can be derived from CD11c<sup>+</sup> bm-DC precursors. Moreover, although we have shown that the function of CD80/CD86 expression on both DC phenotypes appears to lie in the expansion of differentiated T cells, we have not as yet identified the DC signals responsible for Th subset differentiation. However, these results provide new insights into the nature of Th1/Th2 regulation and suggest potential mechanisms for its manipulation. Vaccination strategies based on the differential activation of DC may be particularly useful in situations where persistence of infection or pathology is associated with specific types of immune response as is the case in filariasis and leishmaniasis (Th2) or respiratory syncytial virus infection and endotoxin-induced shock (Th1). In addition, these data provide the basis for novel future approaches to combating diseases such as allergy, autoimmune disorders, or graft rejection where biasing the Th1/Th2 balance could be of therapeutic benefit.

Finally, it is perhaps ironic but gratifying to see that a molecule that appears to aid a pathogenic organism to persist in its parasitized host can be of value in dissecting the mechanisms underlying immune regulation. However, ES-62 may not be alone in this respect, as recently the protozoan pathogens *Plasmodium* and *Leishmania* have been shown to inhibit DC maturation (29, 30). Therefore, DCs may be a common target for infectious agents and hence other "valuable" molecules may await discovery.

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