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Maturation and Activation of Dendritic Cells Induced by Lymphocyte Activation Gene-3 (CD223)¹

Susanne Andreae,* Fabienne Piras,† Nicolas Burdin,† and Frédéric Triebel²*

Lymphocyte activation gene-3 (LAG-3) is an MHC class II ligand expressed on activated T and NK cells. A LAG-3Ig fusion protein has been used in mice as an adjuvant protein to induce antitumor responses and specific CD8 and CD4 Th1 responses to nominal Ags. In this work we report on the effect of LAG-3Ig on the maturation and activation of human monocyte-derived dendritic cells (DC). LAG-3Ig binds MHC class II molecules expressed in plasma membrane lipid rafts on immature human DC and induces rapid morphological changes, including the formation of dendritic projections. LAG-3Ig markedly up-regulates the expression of costimulatory molecules and the production of IL-12 and TNF-α. Consistent with this effect on DC maturation, LAG-3Ig disables DC in their capacity to capture soluble Ags. These events are associated with the acquisition of professional APC function, because LAG-3Ig increases the capacity of DC to stimulate the proliferation and IFN-γ response by allogeneic T cells. These effects were not observed when using ligation of MHC class II by specific mAb. Class II-mediated signals induced by a natural ligand, LAG-3, lead to complete maturation of DC, which acquire the capacity to trigger naïve T cells and drive polarized Th1 responses. The Journal of Immunology, 2002, 168: 3874–3880.

The MHC class II heterodimer (class II)³ is expressed at high levels on the surface of monocyte-macrophages, dendritic cells (DC), and activated B cells. Numerous studies have demonstrated that class II plays an important role as a signal-transducing receptor. For instance, class II-mediated signals affect cell-cell adhesion, Ag presentation, cytokine production, and the expression of costimulatory molecules (1–3). Ligation of class II has also been shown to mediate proliferation/differentiation as well as apoptosis (4–6).

DC are sentinels of the immune system, residing in almost all peripheral tissues (7) in an immature state to allow for optimal Ag uptake. Upon tissue injury or stimulation by infectious agent products such as carbohydrate, LPS, double-stranded viral RNA, or immunostimulatory CpG oligodeoxynucleotides (8–12) DC become activated, migrate to secondary lymphoid organs, and differentiate into mature DC (13, 14). Mature DC express high levels of class I and class II Ags, CD80, CD86, and CD83, produce IL-12, and can prime naive CD4-helper and CD8-cytotoxic T cells (7, 15). The maturation process may also be induced by other stimuli, such as inflammatory cytokines (e.g., TNF-α and IL-1) and cognate CD4+ T cell help, mediated by CD40 ligand (16). Stimulation of CD8+ T cells by DC is achieved after a two-step process: first, DC are induced to mature via CD40/CD40 ligand interaction or by viral infection, and second, this “licensed” DC may directly stimulate cytotoxic T cells (15–18).

We have previously reported that the lymphocyte activation gene-3 (LAG-3), which is embedded in the CD4 locus (19, 20), encodes a protein that binds a nonpolymorphic region of human class II (21, 22) with higher affinities than CD4 (23). LAG-3 (also termed CD223) is expressed in activated NK cells (19, 24) and CD4+ and CD8+ T cells, where it is associated with the CD3/TCR complex at the cell surface (25, 26). LAG-3, like CD4 (27, 28), may oligomerize at the cell surface to interact more efficiently with class II (29). Direct, specific binding of the soluble two- or four-domain LAG-3 extracellular segment has been observed for class II using a human LAG-3Ig fusion protein (22, 29). Finally we showed that LAG-3-transfected tumors in syngeneic mice completely regressed, or their growth was markedly reduced, which was in contrast to the aggressive growth of untransfected cells (30). We also showed that murine LAG-3Ig acts as an adjuvant for Th1 responses and cytotoxic T cell responses to proteinaceous Ags (31). Because murine T cells do not express class II, it seems likely that these responses are induced via a direct effect of LAG-3Ig on class II+ APC.

In this work we study the maturation process induced by LAG-3 of human DC derived from monocytes in the presence of GM-CSF and IL-4. In this model we show that a soluble LAG-3 molecule dramatically induces the maturation of DC. These results are consistent with an important role of class II-mediated activation for the final maturation and licensing of DC.

Materials and Methods

Reagents

Recombinant soluble human LAG-3 molecules were generated by fusing the extracellular domain of hLAG-3 to a human IgG1 Fc portion (22). The resulting recombinant protein was produced in Chinese hamster ovary cells and purified as previously described (22) (M. Subramanyam and M. Tepper, Ares Advanced Technology, Randolph, MA). Preparations contained <1 EU/ml endotoxin as determined by the Limulus amebocyte lysate assay (BioWhittaker, Walkersville, MD). The 17B4 mAb (mlgG1) specific for the LAG-3.1 extracellular domain epitope (the extra loop of Ig-like domain 1) has been previously described (21). The mouse CD32 Ab Fab used in blocking experiments were from Ancell (Bayport, MN).

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Abbreviations used in this paper: class II, MHC class II molecule; DC, dendritic cell; LAG-3, lymphocyte activation gene-3.
Purification of human monocytes and culture of monocytederived DC

Human PBMCs were isolated from venous blood of voluntary healthy donors by Ficoll-Paque density gradient centrifugation (Pharmacia Biotech, Uppsala, Sweden). Monocytes were enriched by aggregation in the cold at a concentration of 50 × 10^6 cells/ml in complete culture medium (RPMI 1640 (BioWhittaker) supplemented with 10% FCS (Life Technologies, Paisley, U.K.), 2 mM glutamine, and 1 mM pyruvate) for 40 min under rotation. The aggregates were separated by sedimentation through 1 ml FCS and depleted of T cells by rosetting on 2-aminoethylisothiouronium bromide (Sigma-Aldrich, St. Louis, MO)-treated SRBC (Bio-Merieux, Marcy l’Etole, France). For this treatment, 2.5 ml SRBC were incubated with 30 ml 5% 2-aminoethylisothiouronium bromide (w/v) for 15 min at 37°C, thoroughly washed, and resuspended in 17.5 ml complete culture medium. Enriched monocytes were then resuspended at 3 × 10^6 cells/ml with 10% of the SRBC suspension and centrifuged on Ficoll-Paque for 25 min at 500 rpm and for 20 min at 2000 rpm to separate the monocyte fraction from SRBC and bound T cells. The resulting preparations were consistently >90% CD14+ as determined by FACS (Elite; Coulter, Miami, FL).

To prepare human immature DC, the purified monocytes were incubated in six-well culture plates (5 × 10^5 cells/3 ml/well) in serum-free RPMI 1640 for 1 h in a humidified incubator at 37°C and 5% CO2. Nonadherent cells were removed, and adherent cells were cultured in 3 ml/well complete culture medium supplemented with 100 ng/ml GM-CSF (Novartis, Rueil-Malmaison, France) and 50 ng/ml IL-4 (R&D Systems, Minneapolis, MN). On days 2 and 4 two-thirds of the culture medium was replaced by fresh medium containing GM-CSF and IL-4, and nonadherent cells were harvested on day 6.

DC stimulation

For maturation experiments, immature DC were resuspended at 1 × 10^6 cells/ml in complete culture medium with cytokines containing either human IgG1 (10 μg/ml; Chemicon, Temecula, CA), human LAG-3Ig (10 μg/ml; Sigma-Aldrich), or different MHC class II Abs (I3, mlG2a; TUD39, mlG2a; TDR31,1, mlG1). After 48 h of culture were harvested and analyzed. In some experiments immature DC were cultured on a monolayer of mouse fibroblasts transfected with human CD32 (provided by C. E. Demeure, Institut Pasteur, Paris, France). Fibroblasts were previously loaded with human IgG1, hLAG-3Ig, or an anti-MHC II Ab (I3) for 30 min at 4°C, thoroughly washed, and fixed with 2% formaldehyde. Treatment with methyl-β-cyclodextrin (10 mM; Sigma-Aldrich) was performed in serum-free medium for 20 min at 37°C before FACS analysis.

Cytofluorometric analysis

To assess the purity and phenotype of cellular preparations, mAbs specific for CD1a, CD3, CD11c, CD14, CD16, CD19, CD54, CD83, MHC I (w6/32), MHC II (I3) (all from Coulter), CD32, CD40, CD64, CD80, CD86 (all from BD Pharmingen, San Diego, CA), and isotype-matched negative controls (Coulter) were used. Cells were incubated with the respective Ab at 10 μg/ml for 30 min at 4°C in PBS/1% BSA and then stained for 30 min at 4°C with FITC-labeled goat anti-mouse F(ab)2 (Coulter). Stained cells were analyzed by FACS using an EPICS Elite cytometer (Coulter).

Assessment of Ag capture

For equilibration 0.5 × 10^6 DC were incubated at 1 × 10^6 cells/ml in complete culture medium for 15 min at 4 or 37°C. FITC-labeled BSA (Sigma-Aldrich) was added at a final concentration of 50 μg/ml, and the cells were incubated for another 30 min to allow capture of the Ag. After thorough washing of the cells with cold medium, fluorescence was measured by FACS analysis. Fluorescence in this assay is indicative of BSA uptake.

Cytokine measurement

Culture supernatants were collected at 48 h and frozen. Commercially available ELISA kits were used according to the manufacturer’s instructions to detect IL-12p40, IL-12p70, and TNF-α in DC culture (R&D Systems). IFN-γ and TNF-α in allostimulation experiments (BioSource, PA). For Scatchard analysis, 23) as expected, LAG-3Ig strongly labels EBV-transformed B cells at 10 μg/ml, as assessed here by measuring mean fluorescence intensity (Fig. 1A). Under these conditions no labeling is observed with 10 μg CD4Ig, a low-affinity binder (23). Despite high surface expression of class

Proliferation assay

Human T cells were purified from PBMC by magnetic separation using CD4+ microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). T cells (1 × 10^6) were mixed in 96-well plates with increasing concentrations of DC (300–10^5 cells) in 200 μl culture medium. On day 4, 1 μCi/well of [3H]thymidine was added, and incorporation of radioactivity was measured after 15 h of incubation (Topcount; Packard Instrument, Meriden, CT). All tests were performed in triplicate.

Results and Discussion

LAG3-Ig shows differences in binding between immature and mature DC

The soluble LAG-3Ig molecule (22) binds class II-positive cells with high affinity (Kd = 60 nM at 37°C on Daudi B cells, as defined by Scatchard analysis) (23). As expected, LAG-3Ig strongly labels EBV-transformed B cells at 10 μg/ml, as assessed here by measuring mean fluorescence intensity (Fig. 1A). Under these conditions no labeling is observed with 10 μg CD4Ig, a low-affinity binder (23). Despite high surface expression of class
II, immature DC were weakly labeled with the same concentration of LAG-3lg (Fig. 1A). DC matured for 48 h with LPS were 10 times more reactive with LAG-3lg, and this increase could not be simply explained by an increase in class II surface expression upon maturation, as the latter was only 2.5 times higher. Similar observations were made when using higher (30 μg/ml) or lower (1 μg/ml) LAG-3lg concentrations (data not shown). The stronger staining of class II expressed on mature vs immature DC observed with LAG-3lg may be related to changes in accessibility or avidity for class II depending upon the clustering of class II in raft microdomains. Rafts, detergent-insoluble glycosphingolipid-enriched membrane microdomains, are preformed 70-nm lipid assemblies rich in kinases and adapter molecules. They serve as platforms for signal transduction and, in clustering, form the TCR-APC contact junction, termed the immunological synapse. LAG-3 was found to be associated with the TCR (25, 26) and to be constitutively present in plasma membrane lipid rafts extracted from human activated T cells based on their insolubility in Triton X-100 and low buoyant density in sucrose gradients (32).

It was thus possible that in the TCR-APC contact junction LAG-3 and class II collaborate in establishing the clustering of raft platforms. Indeed, incubation of immature DC at 4°C for 30 min with a class II-specific mAb, such as I3, which presumably induces an extended clustering of class II+ rafts, leads to increased LAG-3lg binding (data not shown). The partitioning of class II into the raft fraction has been reported to occur in myelomonocytic THP-1 cells following their cross-linking with Abs and to be mandatory for protein tyrosine kinase activation (33). In this work we show that preincubation of immature DC with methyl-β-cyclodextrin, a compound that disrupts protein association with lipid rafts by extracting cholesterol from the plasma membrane (34, 35), dramatically reduced LAG-3lg binding (Fig. 1B). Binding of MHC class II by an mAb was only weakly reduced (Fig. 1B). These observations suggest that LAG-3lg binding indeed depends on the presence of MHC II in raft microdomains. As there may be more class II molecules clustered in organized raft microdomains on mature than immature DC, this difference may explain the stronger binding of their ligand, LAG-3, on mature DC. Also, the restricted binding of LAG-3lg, but not of class II Ab, on raft microdomains on immature DC may explain in part the apparent paradox between weak LAG-3lg binding and its ability to induce DC maturation (see below).

LAG-3 induces morphological changes in immature DC

In previous studies LAG-3lg was found to act as a vaccine adjuvant for the induction of T cell responses to nominal Ag in mice and to induce in vitro cytokine production by monocyte-derived human DC (31, 36). To further analyze the role of LAG-3, monocyte-derived human DC cultured with IL-4 and GM-CSF for 6 days were incubated with soluble LAG-3lg. After 4 h the cells adhered strongly and exhibited an elongated fibroblast-like morphology (Fig. 2B). Following longer incubation times it was revealed by confocal microscopy that regions of the cells detached from the plastic and acquired veils and fine dendrites typical of mature DC (data not shown). In contrast an isotype-matched IgG1 mAb control did not induce morphological changes (Fig. 2A). Interestingly, the purified class II-specific I3 mAb did not have the same effect, but, rather, induced homotypic cell aggregation (Fig. 2C).

LAG-3 induces phenotypic maturation of human DC

These preliminary results prompted us to investigate the capacity of LAG-3lg to induce DC maturation. DC without stimulus or cultured with human IgG1 for 48 h as a control showed the typical phenotype of immature DC (Fig. 3A): low expression of CD40, CD80, and CD86; high levels of class II; and no CD83. As expected, 5 μg/ml LPS induced maturation of DC, with an increase in the expression of cell surface markers CD40, CD80, and CD86, and class II and the expression of CD83. LAG-3lg reproducibly induced similar maturation effects (Fig. 3), with an increase in CD40, CD83, and class II expression comparable to LPS and a somewhat lower increase in CD80 and CD86. On the average, the mean percentage of CD83+ cells with the control IgG1 molecule was 5% at 48 h, while LAG-3lg and LPS increased this to 73 and 87%, respectively. The maturation effect of LAG-3lg was seen by 24 h after stimulation and lasted for >3 days until the cells died (data not shown). Interestingly, the class II-specific I3 mAb (IgG2a) did not induce any shift in the expression level of maturation markers, not even after further cross-linking with a secondary goat anti-mouse Ab (data not shown). Similar observations were obtained with two other pan MHC class II Abs, TU39 (mIgG2a) and TDR31.1 (mIgG1), suggesting that the inability of a given class II Ab to induce DC maturation is not related to a specific class II epitope.

To test the specificity of the effect, LAG-3lg was preincubated for 30 min with saturating amounts of 17B4 before its addition to the DC culture. 17B4 is a mouse mAb that specifically recognizes the first domain of LAG-3 and inhibits binding of LAG-3lg to class II (21). Blocking LAG-3lg binding resulted in dramatic reduction of the induction of maturation by LAG-3lg (Fig. 3), ruling out a nonspecific maturation effect mediated by low level endotoxin contaminants in the LAG-3lg preparation. An irrelevant mlgG1 control had no effect on LAG-3-lg induced DC maturation (data not shown). LAG-3lg contains part of the constant region (hinge-CH2-CH3) of a human IgG1 to dimerize the two LAG-3 exodomains. This Fc region could bind to the low-affinity FcR CD32, the only FcR present on the surface of immature DC (revealed via FACS analysis), and interfere with class II signaling. To rule out any involvement of Fc signaling in the maturation induced by LAG-3lg, we
specitically blocked the FcR on DC with 5 μg/ml anti-CD32 Fab, a concentration shown to block the binding of LAG-3 Ig on murine fibroblast cells expressing high levels of human CD32 molecules following transfection (data not shown). Under these conditions, LAG-3 Ig was still capable of inducing full maturation of DC, indicating that the Fc region of the molecule has no major influence on the maturation effect of LAG-3 Ig (data not shown). Note that neither CD32 Fab alone nor whole Ig coated on plastic had any effect on DC maturation.

Furthermore, we stimulated immature DC with cell-bound LAG-3 Ig, to avoid any binding of the Fc part of LAG-3 Ig to DC. Human IgG1, LAG-3 Ig, or the MHC II Ab I3 was bound via their Fc part to human CD32 receptors on the surface of CD32-transfected murine fibroblasts and used to stimulate immature DC. To exclude any release of bound molecules, we fixed fibroblasts with formaldehyde before stimulation. Confirming our previous observations, membrane-bound LAG-3 Ig was fully capable of inducing DC maturation, as measured by CD83 surface expression (Fig. 3B). Neither IgG1 nor the I3 Ab induced any increase in CD83 expression compared with DC cultured with fibroblasts alone. Therefore, we conclude that LAG-3 Ig-induced maturation of immature DCs is accomplished by class II ligation with its binding domain without any significant involvement of its Fc part. In mouse DC engagement of either FcγRI or FcγRIII induces maturation in an FcR-associated γ-dependent manner (37, 38). However, FcγRI-mediated induction of DC maturation has not been observed in humans, except for FcR specific for IgA, which does induce maturation of human monocyte-derived DC (39). This discrepancy could be due to species differences in FcγR function in mouse and human DC.

Taken together, these data suggest that LAG-3 specifically induces a strong maturation of human DC by binding to its physiological ligand, class II, and that this effect cannot be mimicked by simple aggregation of MHC class II molecules with an Ab.

**DC matured by LAG-3 Ig secrete IL-12 and TNF-α**

Next we examined whether LAG-3 induced functional changes in DC in addition to phenotypic maturation. Maturation is known to induce the secretion of inflammatory cytokines in DC, such as...
IL-12 and TNF-α. Low concentrations of cytokines were detected in the supernatant of immature DC cultured for 2 days with human IgG1 (Table I), but with no difference compared with the negative control (data not shown). Also, a CD1a or class II-specific (I3) mAb did not induce levels of cytokine secretion greater than the negative control (data not shown). In contrast, LAG-3Ig induced the secretion of high levels of both IL-12 (26 ng/ml IL-12p40 \(p < 0.01\), 400 pg/ml IL-12p70 \(p < 0.01\), and 3.5 ng/ml TNF-α \(p < 0.05\)) that could be blocked by addition of LAG-3-specific 17B4 mAb. However, LAG-3 induced secretion was less than that induced by high dose (5 \(\mu\)g/ml) LPS (98 ng/ml IL-12p40 \(p < 0.01\), 1573 pg/ml IL-12p70 \(p < 0.01\), and 36 ng/ml TNF-α \(p < 0.05\)).

We have previously shown that LAG-3 expressed on human T cells synergizes with CD40 ligand to trigger IL-12 and TNF-α production by monocytes (36). In addition, a soluble LAG-3Ig fusion protein was reported to directly stimulate monocyte-derived DC for the production of up to 2 ng/ml TNF-α and IL-12 when using 10 \(\mu\)g/ml LAG-3Ig (36). In the present study the concentration of LIF-12, but not that of TNF-α, in supernatants collected at 48 h was \(\sim10\) times greater (26 ng/ml) and was one-third of the IL-12 concentration that we obtained with high dose LPS. This difference in the level of IL-12 production by monocyte-derived DC in the two studies may be related to differences in differentiation/activation states of immature DC preparations derived from different donors. Our data suggest that this human LAG-3Ig construct may be potentially useful as a vaccine adjuvant, because it induces IL-12 production with rather low levels of TNF-α compared with LPS. Indeed, we reported that a similar mLAG-3Ig fusion protein acted as an adjuvant for Th1 responses and cytotoxic

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**Table I. LAG-3Ig induces the secretion of IL-12 and TNF-α**

<table>
<thead>
<tr>
<th>Cytokine Production</th>
<th>TNF-α (ng/ml)</th>
<th>IL-12p40 (ng/ml)</th>
<th>IL-12p70 (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1 control</td>
<td>0.037 ± 0.21</td>
<td>2.1 ± 1.7</td>
<td>72 ± 41</td>
</tr>
<tr>
<td>LAG-3Ig</td>
<td>3.5 ± 0.75</td>
<td>26 ± 6.4</td>
<td>400 ± 153</td>
</tr>
<tr>
<td>LAG-3Ig + 17B4</td>
<td>0.062 ± 0.45</td>
<td>0.71 ± 1.9</td>
<td>93 ± 24</td>
</tr>
<tr>
<td>LPS</td>
<td>36 ± 8.9</td>
<td>98 ± 14</td>
<td>1573 ± 843</td>
</tr>
</tbody>
</table>

*Immature DC were incubated with either 10 \(\mu\)g/ml IgG1 (control), 10 \(\mu\)g/ml LAG-3Ig, 10 \(\mu\)g/ml LAG-3Ig preincubated with 20 \(\mu\)g/ml LAG-3-specific mAB (17B4), or 5 \(\mu\)g/ml LPS. After 2 days, cell culture supernatants were collected for assessment of IL-12 and TNF-α by ELISA. Results are means ± SD of at least three experiments.*

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FIGURE 4. Maturation by LAG-3Ig inhibits Ag capture of DC. Human DC incubated for 48 h with IgG1 at 10 \(\mu\)g/ml (filled histograms), LAG-3Ig at 10 \(\mu\)g/ml (gray histogram), or LPS at 5 \(\mu\)g/ml (open histogram) were incubated for 30 min at 4 or 37°C with 50 \(\mu\)g/ml BSA-FITC, and fluorescence was measured by FACS analysis. Similar results have been obtained in five additional experiments.

FIGURE 5. Maturation by LAG-3Ig increases allostimulatory capacities. Immature DC cells were treated with IgG1 (5 \(\mu\)g/ml), LAG-3Ig (5 \(\mu\)g/ml), or TNF-α, IL-1β, and LPS (10 ng/ml each) for 2 days. Increasing numbers of these cells were then incubated with 1 \(\times\) 10^6 allogeneic T cells for 4 days. A, Proliferation of T cells was measured by the incorporation of radioactive [3H]thymidine. The mean ± SD of triplicate determinations of one representative experiment of three are indicated. Coculture supernatants were assessed for the cytokines IFN-γ (B) and TNF-α (C) by ELISA.
T cell responses in mice immunized with either a particulate (HBsAg) or a soluble (albumin) Ag (31), with no toxicity when up to 100 µg LAG-3lg were injected s.c. at the site of the vaccine.

Maturation by LAG-3lg inhibits Ag capture of DC
Because mature DC lose their capacity for uptake and processing of Ag, we examined the capture of FITC-labeled BSA by LAG-3 matured DC (Fig. 4). At 4°C cytoskeletal rearrangements and cell metabolism are inhibited such that DC are incapable of capturing Ag. However, at 37°C the two control populations (no stimulus or human IgG1) showed significant uptake of the BSA-FITC test Ag revealed by an increase in green fluorescence. Cross-linking of class II with specific mAb did not alter Ag uptake (data not shown). In contrast, LPS induced a loss of this capacity (Fig. 4). DC that were matured by LAG-3lg also completely lost the ability to capture Ag and did not take up more Ag than the control population at 4°C (Fig. 4). This inhibitory effect on Ag capture was reversed by preincubation of LAG-3lg for 30 min with saturating amounts of 17B4 LAG-3-specific mAb (data not shown). Therefore, LAG-3lg is a potent maturation stimulus for DC functions such as cytokine secretion and Ag uptake, which cannot be replicated by simply cross-linking class II with specific mAb.

LAG-3lg induces strong allosstimulatory capacities of DC
Maturation and activation of DC are associated with the acquisition of professional APC function. Mature DC migrate to the lymph nodes, where they are potent stimulators of T cell responses. Therefore, we examined the efficiency of LAG-3-lg matured DC to activate T cells in a MLR with purified CD4+ T cells from a different donor. Clearly, LAG-3 matured DC were potent inducers of T cell proliferation, as the response was as intense as with positive control cells incubated with TNF-α, IL-1β, and LPS (Fig. 5A), while immature DC or cells incubated with the negative control human IgG1 were poor activators. Indeed, 10-fold more immature DC were required to activate T cells compared with LAG-3 matured DC (Fig. 5A). These results were confirmed by evaluating IFN-γ and TNF-α secretion by ELISA. LAG-3-lg matured DC secreted cytokine levels comparable to LPS maturation (Fig. 5, B and C). These data indicate that LAG-3lg is capable of inducing a fully functional maturation of human monocyte-derived DC.

Concluding remarks
Our in vitro data demonstrate that a human LAG-3lg protein causes maturation/activation of monocyte-derived DC, converting them into professional APC able to initiate productive T cell immune responses. These findings are in line with our previous in vivo experiments with immunized mice and represent a further step toward the use of this protein as an adjuvant for subunit vaccines. It also indicates that class II plays an important role as a signal-transducing receptor on immature DC.

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