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FcαRI-Mediated Inhibition of IL-12 Production and Priming by IFN-γ of Human Monocytes and Dendritic Cells

Marylène Lecocq,* Bruno Detry,* Amélie Guisset,* and Charles Pilette*,†

We showed that IgA induces IL-10 in monocytes and dendritic cells. Because reciprocal inhibition exists between IL-10 and IL-12, we explored whether IgA could regulate this other immunoregulatory cytokine. In human monocytes and monocyte-derived dendritic cells preincubated with IFN-γ before stimulation by LPS, suppression of p40 and IL-12p70 production was observed upon IgA treatment during IFN-γ priming. Washout experiments and inhibition of IFN-γ–induced CXCL10 (IP-10) and FcγRI (CD64) indicated that inhibition by IgA occurred at both the LPS and IFN-γ levels. Inhibition was not affected by blockade of IL-10 or MAPK but involved FcαRI/CD89-mediated suppression of STAT1 phosphorylation. These data indicate that FcαRI ligation on human monocytes and dendritic cells inhibits IL-12 expression and type 1 activation by interfering with STAT1 activation. *The Journal of Immunology*, 2013, 190: 2362–2371.

Antigen presenting cells, including dendritic cells (DCs), are master regulators of immune responses through their unique capacity to both recognize danger signals and subsequently activate T cells. The latter follows cognate interactions between APCs and TCRs (signals 1 and 2), as well as secretion of immunoregulatory cytokines (signal 3) that imprint the polarization of the T cell response. In particular, APC-derived IL-12 represents the major signal for T cells to differentiate into Th1 cells that produce IFN-γ. IL-12 can also trigger T cells to express TNF-α, IL-2, IL-3, and GM-CSF (1), and it elicits the generation of CD8$^+$ T cells and activated NK cells expressing cytotoxic effector molecules (e.g., perforin and granzymes). In addition, IL-12, along with IFN-γ, antagonizes Th2 differentiation.

IL-12p40 is secreted as monomers and homodimers in large excess over IL-12p70 (the bioactive form of IL-12) both in vitro and in vivo (2, 3). The *p40* gene is highly inducible by microbial products, and its promoter contains sites for NF-κB (4, 5), AP-1 (5), IRF-1 (6), and Ets family members (7, 8). Unlike p40, p35 is regulated both transcriptionally and posttranscriptionally and, despite constitutive p35 mRNA synthesis, little or no protein is secreted because of the presence of an inhibitory ATG motif in the 5′-untranslated region (1, 9).

IFN-γ is located both up- and downstream of IL-12, enhancing IL-12 production while concomitantly suppressing IL-10 in response to TLR stimulation (10). IFN-γ produced by activated NK cells or CD8$^+$ T cells can skew the development of DCs toward a type 1 phenotype with high IL-12 expression (11). In human monocytes, production of bioactive IL-12 (p70) requires a combination of stimuli, such as LPS and IFN-γ. IFN-γ ligation of its receptor subunits (IFNGR-1 and -2) triggers recruitment of JAK enzymes (JAK-1 and -2), which are transphosphorylated and, in turn, phosphorylate the IFNGR (1) on tyrosines within the intracellular domain that generate docking sites for STAT1. Recruited STAT1 is phosphorylated by JAK2 and then homo- or heterodimerizes and translocates into the cell nucleus where it binds to specific promoters of IFN-γ–dependent genes to induce their transcription (12).

IgA is the predominant Ig at mucosal surfaces, and it can regulate myeloid leukocytes through the FcαRI (CD89). Thus, IgA is mainly produced in mucosal tissues and is endowed with both suppressive and activating effects [e.g., according to the level of receptor cross-linking (13)] during the effector phase of inflammatory responses. Other properties include neutralization of pathogens and poor proinflammatory capacity, such as for complement activation. In contrast, it remains poorly known whether IgA, abundantly present in subepithelial tissues and blood, can also regulate the initiating steps of immune responses. We previously showed that IgA can induce IL-10 expression in human monocytes (14) and monocyte-derived DCs (MD-DCs) (15). Because reciprocal inhibition may occur between autocrine IL-10 and IL-12 (16), the aim of the current study was to explore whether IgA could also regulate IL-12 expression in human monocytes and MD-DCs, as well as to investigate the molecular mechanisms of this modulation, including the role of autocrine IL-10 and intracellular signaling.

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Abbreviations used in this article: DC, dendritic cell; MD-DC, monocyte-derived dendritic cell; RT-qPCR, RT-quantitative PCR.

Materials and Methods

Study population

Peripheral blood was collected from volunteer donors. All donors were healthy and exhibited no sign of active infectious or atopic disease. The study was approved by the Ethics Committee of the Cliniques Universitaires Saint-Luc, and all subjects gave written informed consent.

Isolation of monocytes and generation of DCs

Blood cells were layered over a Ficoll gradient (Lymphoprep; Axis-Shield, Oslo, Norway). After centrifugation (800 × *g*, 30 min), PBMCs were isolated, washed, and resuspended in PBS ([pH 7.2] supplemented with 0.5% BSA and 2 mM EDTA). PBMCs were incubated with magnetic beads conjugated with monoclonal mouse anti-human CD14 Abs (Miltenyi

Biotec, Bergisch Gladbach, Germany) on ice for 15 min. After washing, the cells were applied onto a column placed in the magnetic field of a MACS separator (Miltenyi Biotec). After elimination of negative cells, the column was removed from the separator, and the CD14^{+} cells (monocytes) were collected and washed twice in RPMI 1640 (1:1; Lonza, Verviers, Belgium) culture medium containing 10% heat-inactivated FCS, 2 mM L-glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (all from Lonza).

For generation of myeloid DCs, monocytes purified by adherence were treated according to the protocol described by Sallusto and Lanzavecchia (17). Monocytes were differentiated into immature MD-DCs at 1×10^6/ml in six-well flat-bottom culture plates in RPMI 1640 in the presence of GM-CSF and IL-4 (both 20 ng/ml; R&D Systems, Oxfordshire, U.K.) for 6 d. At the end of the culture, MD-DCs were harvested, washed, and used for subsequent experiments.

Monocyte and MD-DC activation

Monocytes or MD-DCs (1×10^6 cells) were cultured in 12-well flat-bottom plates in 1 ml (or alternatively for dose-response experiments, 2×10^5 cells in 96-well culture plates in 200 μl) RPMI 1640 culture medium. Cells were incubated in triplicate at 37°C, 5% CO_2 for 24 h with IFN-γ (20 ng/ml; R&D Systems) in the presence or absence of dimeric IgA (1 mg/ml), prepared from myeloma serum in our laboratory; unless otherwise indicated, IgA treatment refers to this myeloma IgA. In addition, normal monomeric IgA prepared from a pool of normal human serum was used (at a concentration of 1 mg/ml). Culture was then carried out with medium or with LPS (1 μg/ml; Sigma, St. Louis, MO) at 37°C, 5% CO_2 for another 24 h. The inhibitors or Abs were added 1 h (at 4°C) before cell stimulation; the concentration used was 50 μM for PD-98059, SB-203580 (Biomol International, Plymouth Meeting, PA), SP-600125 inhibitors (A.G. Scientific, San Diego, CA), and AG-490 inhibitor (Sigma). Cross-linking Ab to CD89 (clone MIP7c; Pierce, Rockford, IL) and control mouse IgG (kind gift from Prof. J.P. Coutelier, de Duve Institute, Brussels, Belgium) were used at 10 μg/ml. In selected experiments, monocytes were preincubated at 4°C on ice with IgA and F(ab′)₂ fragments [prepared using ImmunoPure F(ab′)₂ preparation kit from Pierce of affinity-purified goat ACP17 anti-human IgA, previously generated in our laboratory] separately (twice for 30 min), with a gentle wash between incubation before IFN-γ priming and LPS stimulation. After 48 h, cells were harvested for phenotypic analysis by flow cytometry, optical microscopy (using a Axiovert-

40 microscope; Zeiss, Jena, Germany), or RT-PCR analyses, and supernatants were collected and frozen (−20°C) until cytokine assays.

Flow cytometry

Monocyte and myeloid DC purity was assessed by FACS analysis using the following surface markers: CD1a-FITC, HLA-DR-PerCP, CD80/B7.1-PE, CD86/B7.2-PE, CD83-FITC, CD11c-allophycocyanin, and CD14-FITC (all from BD Biosciences, San Diego, CA). CD89/FcαRI and CD64/FcγRI (BD Biosciences) were also assessed before/after monocyte stimulation (as a marker of IFN-γ–induced monocyte activation). Immunostaining was carried out in PBS–2% FBS (Lonza) before washing and fixation in 0.5% formaldehyde (Sigma). Acquisition was carried out for monocytes on 10,000 CD14^{+}-gated cells/condition, using a FACS Canto II (BD Biosciences). Results are expressed as mean fluorescence intensity, corrected for the isotypic control.

Cytokine assays

IL-12p40 and IL-12p70 were measured by sandwich ELISA in the cell culture supernatants using paired Abs (R&D Systems), with a sensitivity of 16 pg/ml for each assay. CXCL-10/IP-10 and CCL22/MDC were also quantified by ELISA (R&D Systems).

Quantitative RT-PCR for p40, IL-12p35, and STAT1

Total RNA was isolated using the RNeasy Plus Mini kit (QIAGEN, Hilden, Germany). Total RNA was reverse transcribed with a RevertAid Reverse Transcriptase kit (Fermentas, St. Leon-Rot, Germany) with 0.3 μg random hexamer (Invitrogen, Carlsbad, CA) and 1 mM each deoxynucleotide triphosphate (Invitrogen), following the manufacturer's protocol, in a thermocycler (Applied Biosystems, Carlsbad, CA). The expression levels were quantified by real-time quantitative PCR with the iCycler IQ5 PCR (Bio-Rad, Hercules, CA). The reaction mix contained 5 μl cDNA diluted 10-fold, 400 nM each primer (p40 reverse: 5′-AACCTAACTGCAGGGCACAG-3′; p40 forward: 5′-CGGTCATCTGCCGCAA-3′; p35 reverse: 5′-ATTCTA-GAGTTTGTCTGGCCTT-3′; p35 forward: 5′-CACTCCAGACCCAG-GAATGTT-3′; STAT1 reverse: 5′-GTGTTCTCTGTTCTGCAAGGTTT-3′; STAT1 forward: 5′-TGAAGGACAAGGTTATGTGTATAGAGC-3′; RPS18 reverse: 5′-TGATCACACGTTCCACCTCAT-3′; RPS18 forward: 5′-TGTGGGCCGAAGATATGCT-3′), and 2× iQ SYBR Green SuperMix (Bio-Rad) in a final volume of 20 μl. The cycling conditions were 95°C for

FIGURE 1. Inhibition by IgA of p40 and IL-12p70 production by human monocytes primed by IFN-γ and stimulated by LPS. Dose response of p40 (**A**, n = 3) and IL-12p70 (**B**, n = 2) production upon LPS stimulation following priming with increasing concentrations of IFN-γ from 5 to 80 ng/ml. Effect of IgA on monocyte production of p40 (**C**, n = 8) and IL-12p70 (**D**, n = 9) after IFN-γ and/or LPS stimulation. (**E** and **F**) Effect of IgA on monocyte expression of p40 mRNA and IL-12p70 mRNA by RT-qPCR, corrected for RPS18. Results depict mean ± SEM. **p < 0.01, ***p < 0.001.
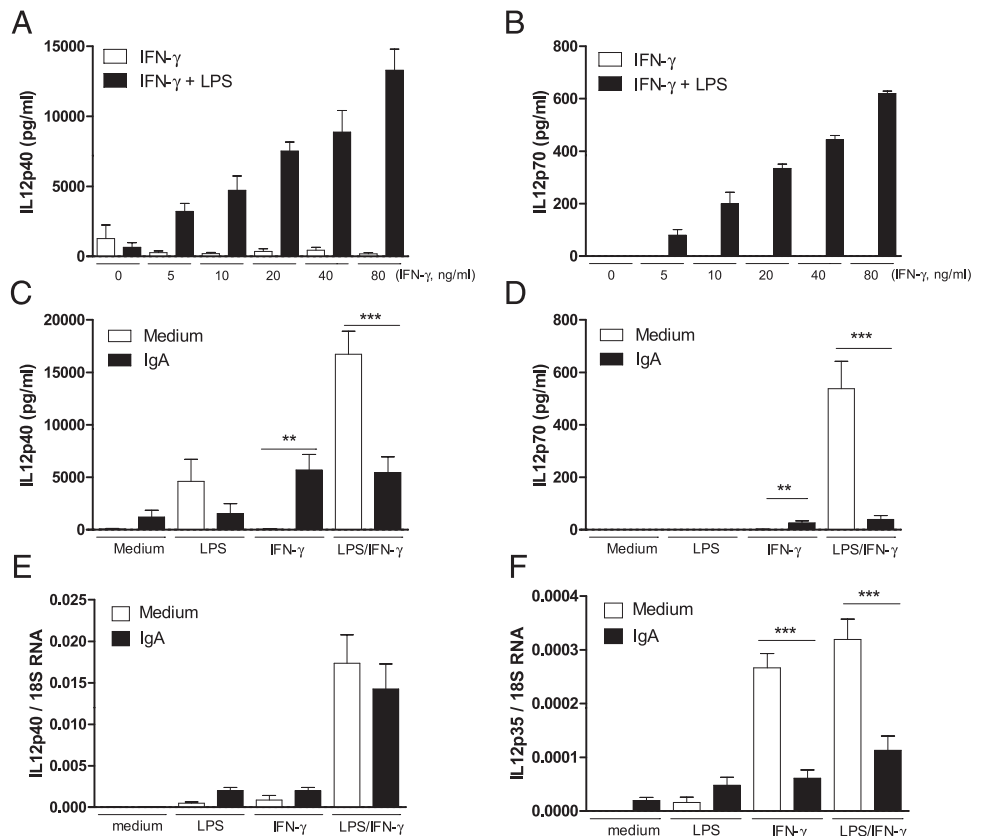
3 min, followed by 40 cycles of 95°C for 15 s and 60°C for 30 s. A melting curve analysis was performed to control the specificity of the amplification products. Moreover, the negative controls (water and RT) showed no amplification. Samples were run in duplicates, and the copy number was calculated from a standard curve. The standard curve and data analysis were produced using Bio-Rad iQ5 Software.

Western blot for p-MAPKs and STAT1

Monocytes were stimulated by IFN-γ/LPS, with or without IgA, as described above, in 12-well plates at 2×10^6 cells/well in duplicate. Cells were subsequently lysed for 30 min on ice in 150 μl RIPA lysis buffer (50 mM Tris-HCl [pH 7.4], 0.25% sodium deoxycholate, 150 mM NaCl, 1 mM EDTA, 1 mM PMSF, 1 mM Na3VO4, 1 mM NaF). Lysates were clarified by centrifugation at 4°C for 10 min at $10,000 \times g$. Equal amounts of lysates were separated by 12% SDS-PAGE and electrotransferred onto a nitrocellulose membrane and subjected to immunoprobing using Abs to phosphorylated or unphosphorylated MAPKs ERK1/2, p38-MAPK, JNK, and STAT1 (1:1000, rabbit polyclonal Ab; Cell Signaling Technology, Danvers, MA) or GAPDH (1:5000, rabbit polyclonal Ab; Sigma). Secondary Abs consisted of HRP-conjugated goat IgG anti-rabbit IgG (1:2000; Cell Signaling Technology). Immunoreactive bands were developed using chemiluminescence (Amersham ECL; GE Healthcare, Buckinghamshire, U.K.) and detected with a Chemidoc XRS apparatus (Bio-Rad). The intensity of each band was measured with the densitometry program Quantity One (Bio-Rad).

Statistical analysis

Data are presented as mean ± SEM, unless otherwise stated. Comparisons were carried out using the two-tailed paired *t* test, with the Bonferroni correction applied for multiple comparisons, with GraphPad Prism 4.0 (GraphPad Prism, San Diego, CA). The *p* values < 0.05 were considered statistically significant.

Results

Inhibition of IL-12 production by IgA

A sharp priming effect of IFN-γ was confirmed on p40 (Fig. 1A) and IL-12p70 release (Fig. 1B) by LPS-activated monocytes. In this system, highly significant reductions in IL-12 release by activated monocytes were observed following pretreatment (concomitantly with IFN-γ) with IgA (Fig. 1C, 1D), particularly on IL-12p70. Thus, although IgA exhibited a stimulating effect that was amplified by IFN-γ, a striking inhibitory effect of IgA was observed on IL-12p40 and p70 following combined IFN-γ priming and LPS stimulation (Fig. 1C, 1D). At the gene level, although marginal changes in p40 mRNA were observed in IgA-treated monocytes (Fig. 1E), IL-12p35 mRNA was very significantly reduced by IgA following IFN-γ or IFN-γ/LPS stimulation (Fig. 1F).

IgA-mediated suppression of the priming effect of IFN-γ on IL-12 production

To evaluate whether suppression by IgA involved interference with LPS and/or IFN-γ pathways, a washout experiment was carried out. Monocytes were washed after IFN-γ preincubation (with or without IgA), before LPS stimulation (Fig. 2). In its absence during LPS incubation (washout), IgA preincubation could not prevent the effect of LPS, whereas IgA's effect on IFN-γ priming was maintained (Fig. 2B versus 2A). These results suggested that, to inhibit IL-12, IgA could target, at least in part, IFN-γ signaling.

Inhibition by IgA of IFN-γ–dependent signals

The Th1-related chemokine CXCL10 (IP-10) was assessed as another IFN-γ–dependent signal. CXCL10 was induced by IFN-γ stimulation, as well as by LPS to a much lower extent; both effects were independently suppressed by preincubating cells with IgA (Fig. 2C). In contrast, the Th2-related chemokine CCL22 (MDC) was induced upon IgA treatment of monocytes to a greater extent than with LPS (Fig. 2D).

Dose-response experiments showed that inhibition occurs at IgA concentrations from 0.1 mg/ml and that IL-12p70 was more
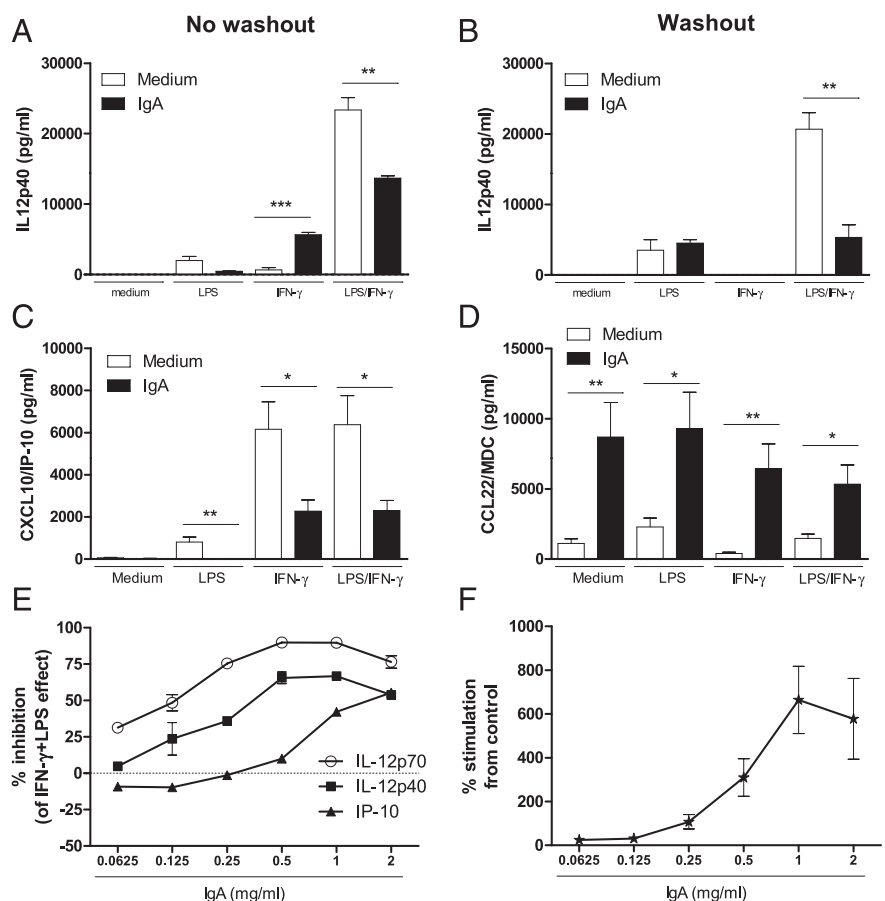

FIGURE 2. Dose-response effect of IgA on IFN-γ priming for IL-12 and CXCL10/IP-10 production. Effect of IgA on monocyte production of p40 without (**A**, *n* = 3) or after (**B**, *n* = 3) washout following IFN-γ priming. Effect of IgA on CXCL-10 (**C**, *n* = 8) and CCL22/MDC (**D**, *n* = 6) production. (**E**) Inhibition percentage of p40, IL-12p70, and CXCL10/IP-10 in IFN-γ–primed LPS-stimulated monocytes by various concentrations of IgA (*n* = 2). (**F**) Effect of different IgA concentrations on CCL22/MDC release (*n* = 2). Results depict mean ± SEM. **p* < 0.05, ***p* < 0.01, ****p* < 0.001.

FIGURE 3. Inhibition by normal serum IgA of p40, IL-12p70, and CXCL10/IP-10 production by human monocytes primed by IFN-γ and stimulated by LPS. Effect of normal IgA on monocyte production of p40 (**A**, *n* = 3), IL-12p70 (**B**, *n* = 3), and CXCL10/IP-10 (**C**, *n* = 3) after IFN-γ and/or LPS stimulation. (**D**) Effect of different concentrations of normal serum IgA on CXCL10/IP-10 production by monocytes primed by IFN-γ and stimulated by LPS (*n* = 3). Results depict mean ± SEM. *$p < 0.05$.
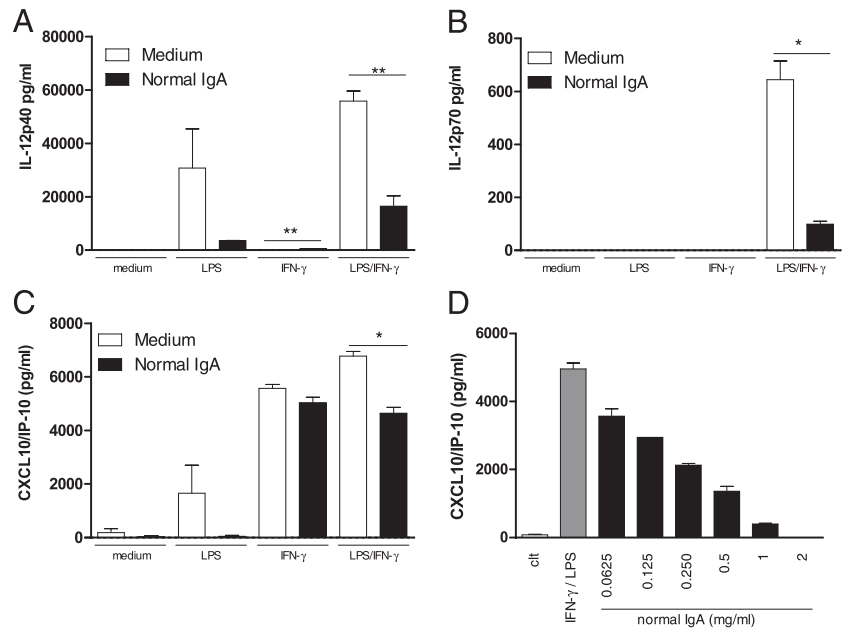
sensitive to inhibition by IgA than was p40 or CXCL10 (Fig. 2E), whereas upregulation of CCL22 required higher concentrations (≥0.5 mg/ml) (Fig. 2F).

Inhibition of IL-12 and CXCL10 by different forms of human IgA

To exclude a particular effect of myeloma IgA, inhibition was confirmed using IgA purified from a pool of normal human serum. Monocytes were incubated with IFN-γ and normal serum IgA before LPS stimulation. Inhibition of IL-12 in monocytes primed by IFN-γ/LPS (Fig. 3A, 3B) was similar to that observed with myeloma IgA (Fig. 1C, 1D). The suppression of IFN-γ–induced CXCL10/IP-10 was also confirmed with normal IgA (Fig. 3C), in a dose-dependent manner (Fig. 3D).

IgA-mediated inhibition of IL-12 and CXCL10/IP-10 is dependent on FcαRI ligation

Cross-linking of IgA receptors using IgA, followed by anti-IgA Ab, reproduced the inhibitory effect of IgA on IL-12 and CXCL10/IP-10 in IFN-γ–primed LPS-stimulated monocytes (Fig. 4A). FcαRI/CD89 was then assessed as the putative receptor mediating these effects of IgA. FcαRI cross-linking recapitulated the suppressive effect of IgA on IL-12p40, in contrast to the nonsignificant effect with control mouse IgG (Fig. 4B). These results indicated that the effect of IgA on IL-12 is dependent on the myeloid FcαRI/CD89 receptor.

IgA-mediated inhibition of type 1 monocyte activation

In these cultures, the morphological features of monocytes activated by LPS and/or IFN-γ were affected by IgA treatment. Thus,

activation by LPS and IFN-γ resulted in the formation of cell clusters, an effect largely inhibited in the presence of IgA (Fig. 5A). Effects of IgA on markers of cell activation were then assessed by flow cytometry. The presence of FcαRI was first confirmed on freshly purified monocytes, and it was upregulated following culture in the presence of IgA (Fig. 5B). FcγRI/CD64 was induced upon IFN-γ treatment of monocytes, and expression of the Ag presentation–related surface molecules HLA-DR, CD80, and CD86 was strongly upregulated upon combined stimulation with LPS and IFN-γ (Fig. 5C). Both IFN-γ–induced CD64 and LPS/IFN-γ–driven upregulation of HLA-DR, CD80, and CD86 were inhibited in the presence of IgA. These morphological and phenotypic effects further indicated that IgA suppresses IFN-γ–driven monocyte activation.

Inhibition of IL-12 release by IgA in DCs

As expected, in MD-DCs, LPS stimulation was sufficient to induce IL-12 production, including bioactive IL-12p70, showing that DCs are much less dependent on a second signal to produce this cytokine than are monocytes. IgA was also able to inhibit IL-12 release by MD-DCs stimulated by LPS or IFN-γ/LPS (Fig. 6A, 6B).

IgA-mediated inhibition of IL-12 is independent from IL-10

To address the role of autocrine IL-10, which is induced by IgA in monocytes (14) and DCs (15) and can inhibit IL-12 production (16), DCs were pretreated with a blocking mAb to human IL-10Rβ. Blockade of the IL-10 pathway induced a slight in-
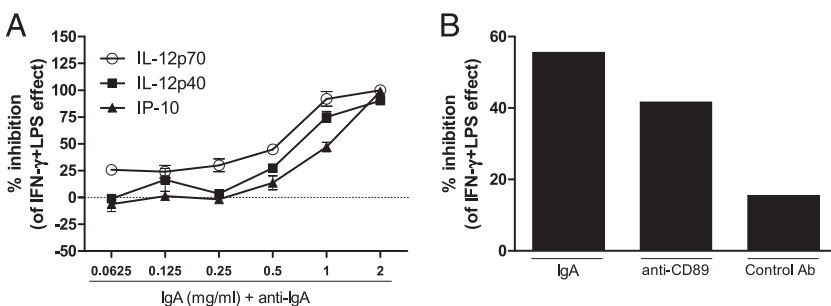

FIGURE 4. Effect of IgA/FcαR cross-linking on monocyte IL-12 production, following priming by IFN-γ and LPS stimulation. (**A**) Inhibition percentage of p40, IL-12p70, and CXCL10/IP-10 in IFN-γ–primed LPS-stimulated monocytes by various concentrations of IgA + F(ab′)₂ anti-IgA fragments (*n* = 2). (**B**) Inhibition percentage of p40 by monocytes after IFN-γ priming and LPS stimulation, in the presence of IgA or cross-linking anti-FcαRI/CD89 Ab, compared with control mouse IgG (data are representative of two separate experiments).
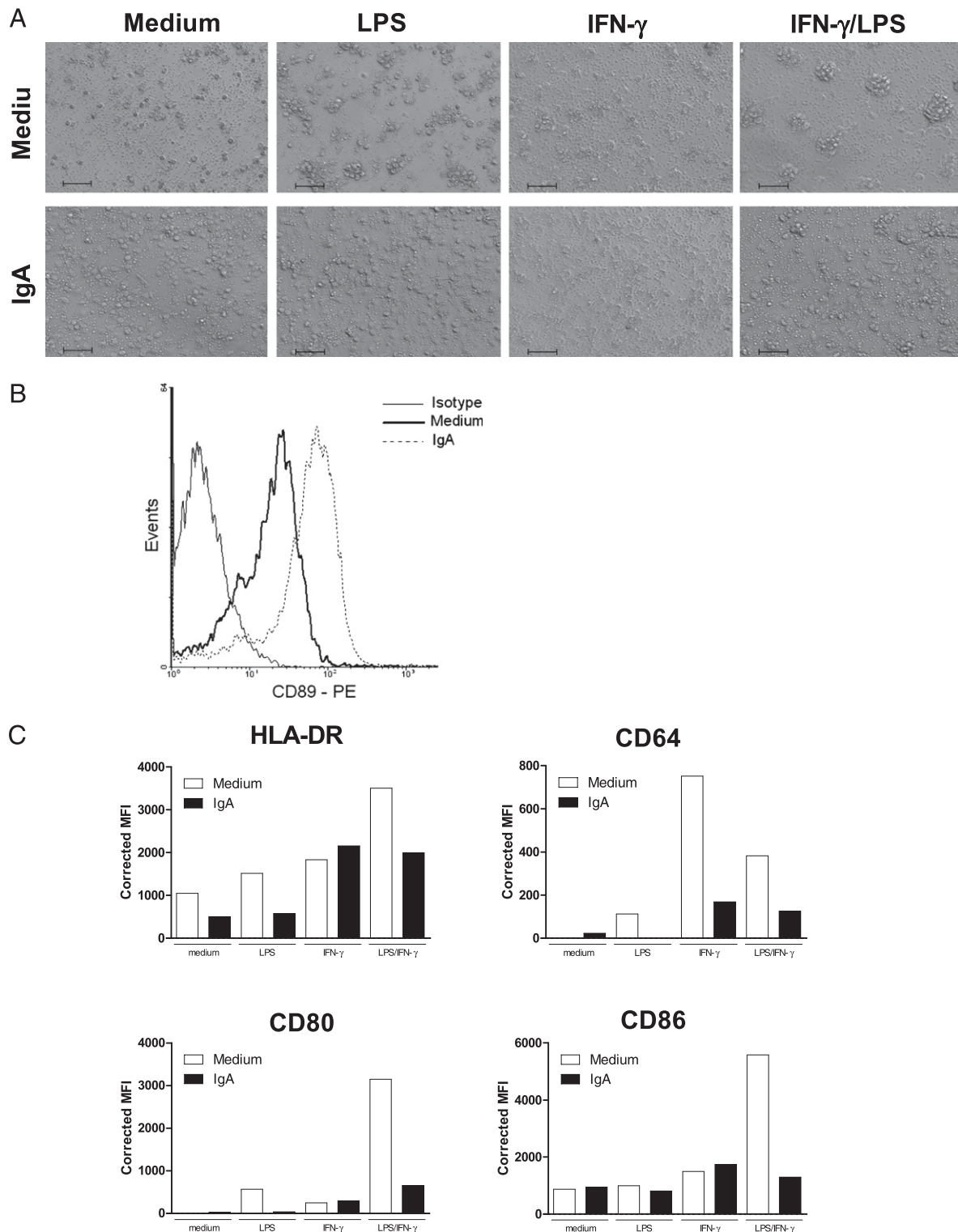
FIGURE 5.   Effects of IgA on phenotypic markers of monocyte activation. (**A**) Morphological effect of IgA on monocyte appearance in culture following IFN-γ and/or LPS stimulation. Monocytes were assessed by inverted optical microscopy at the end of the culture with IgA, with/without activation by IFN-γ and/or LPS. Scale bars, 50 μm. (**B**) Expression of FcαRI (CD89) on monocytes at baseline or after incubation with IgA. (**C**) Regulation by IgA of activation markers of monocytes (i.e., HLA-DR, FcγRI/CD64, CD80, and CD86). Data are representative of three independent experiments and are expressed as mean fluorescence intensity (MFI), corrected for the isotype control.

crease in p40 and IL-12p70 production by DCs (Fig. 6C, 6D), as expected, according to reciprocal inhibition between IL-10 and IL-12, whereas isotype control had no effect. However, IL-10Rβ blockade did not affect the inhibitory effect of IgA on IL-12 production by DCs (Fig. 6C, 6D) or fresh monocytes (data not shown).

IgA-mediated inhibition of IL-12 is independent from MAPK regulation

MAPKs were assessed as intracellular signals potentially targeted by IgA to inhibit IL-12 production in monocytes/DC. First, the requirement of ERK, p38, and JNK for IL-12 (p40, p70) production was addressed using selective inhibitors of these pathways (Fig. 7A,

FIGURE 6. Inhibition by IgA of p40 and IL-12p70 production by MD-DCs and role of autocrine IL-10. Effect of IgA on MD-DC production of p40 (**A**, $n = 4$) and IL-12p70 (**B**, $n = 4$) after IFN-γ and/or LPS stimulation. Effect of blocking IL-10Rβ on IgA-driven inhibition of p40 (**C**, $n = 2$) and IL-12p70 (**D**, $n = 2$) following IFN-γ/LPS stimulation. Results depict mean ± SEM. *$p < 0.05$.
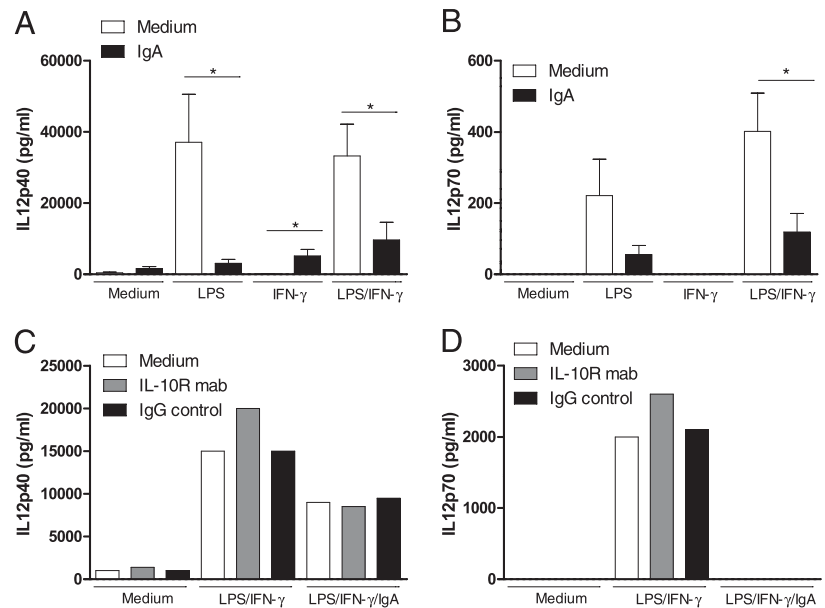
7B), which were confirmed to suppress the target MAPK without inhibitory effect on the two others, as well as to inhibit the LPS-induced production of TNF and IL-10 (data not shown). Although MAPK inhibitors had differential effects on p40 and IL-12p70 in IFN-γ/LPS-activated monocytes (Fig. 7A, 7B), IgA had no inhibitory effect on LPS-induced p38 and JNK phosphorylation and a very modest effect on ERK (Fig. 7C). Thus, IgA had no significant effect on MAPKs that could underlie its inhibitory effect on IL-12.

Inhibition of STAT1 activation by IgA

Because JAK/STAT molecules are critical to IFN-γ activity (18), a potential interference between FcαR and IFN-γ pathways was investigated at this signaling level. Using a selective JAK2/STAT1 inhibitor (AG-490), we first confirmed the requirement of this pathway for IL-12 production in activated monocytes. JAK2/STAT1 blockade resulted in a failure of LPS alone and the IFN-γ/LPS combination to elicit IL-12p40 release (Fig. 8A).

A marked reduction in LPS-induced STAT1 phosphorylation was observed in IFN-γ–primed monocytes following IgA preincubation (Fig. 8B). In addition, a mild, but consistent, reduction in total STAT1 was also observed following IgA pretreatment (Fig. 8B).

Moreover, inhibition of STAT1 phosphorylation was also observed upon IgA treatment following short-term IFN-γ stimulation (Fig. 8C). This latter effect required preincubation with IgA for 24 h and was not observed when IgA was added only 1 h before IFN-γ (data not shown), and it also resulted in a decrease in total STAT1 (Fig. 8C). Finally, a reduction in transcription of *STAT1* by IgA was confirmed in LPS-activated monocytes by RT-quantitative PCR (RT-qPCR) for STAT1 mRNA (Fig. 9).

Discussion

IL-12 is the major Th1-polarizing factor, produced upon TLR-mediated activation of DCs, to elicit IFN-γ responses following cognate interactions with T cells. To our knowledge, the present study shows for the first time that, in human monocytes and MD-DCs, IgA suppresses IL-12 induction upon IFN-γ and LPS stimulation. Suppression involved interference with both LPS and IFN-γ pathways, as indicated in washout experiments and by concomitant inhibitory effects of IgA on other IFN-γ–dependent

signals, such as chemokine IP-10/CXCL10, CD64, and Ag presentation–associated molecules. IgA-mediated inhibition of IL-12 expression did not involve autocrine IL-10 or modulation of MAPKs, but it involved interference, via the myeloid FcαRI/CD89 receptor, with the activation of STAT1 as the critical intracellular signal in LPS- and IFN-γ–activated monocytes.

Inhibition of IL-12 was described previously with various mediators, through various mechanisms. Vitamin D3 inhibits IL-12 expression via inhibition of binding to NF-κB site (19). Glucocorticosteroids also suppress LPS-induced IL-12p40 transcription by inhibiting c-Jun (5), which signals through AP-1 and NF-κB (5, 20). PGE$_2$ (21) and catecholamines (activating the β2-adrenoreceptor) (22) inhibit IL-12 production in human monocytes through increased cAMP. In addition to these factors, cross-linking of FcγR by IgG immune complexes renders murine macrophages refractory to IL-12 production (23, 24), and reciprocal alteration in the production of IL-10 and IL-12 by IgG immune complexes has been observed. It was shown that the effect of IgG on IL-12p40 involves PI3K signaling and inhibition of NF-κB binding (25). It was also described that murine macrophages infected with the Ross River virus in the presence of LPS and subneutralizing concentrations of IgG Abs display reduced IFN-γ and TNF-α responses, which correlated with reduced STAT1 and NF-κB activation, respectively (26); however, this was not mediated through FcγR, because nonviral (zymosan) immune complexes failed to suppress STAT1 and NF-κB binding.

A strong inhibition of IL-12 expression was observed with IgA on IL-12p70 at the protein and gene (*p35*) levels in LPS+IFN-γ–treated monocytes, whereas inhibition of p40 was observed to a lower extent and mainly at the protein level. In addition, a mild induction of IL-12p70 is observed with IgA in IFN-γ–treated cells in contrast to p35mRNA inhibition, further suggesting that IgA operates through both transcriptional and posttranscriptional mechanisms. In addition to IL-12 inhibition, we show that IgA is able to inhibit CXCL10/IP-10, a major chemokine for Th1 cells. Moreover, it can also concomitantly induce CCL22/MDC production (a prototypical Th2 chemokine), further suggesting that IgA could display pro-Th2 effects during developing immune responses. Considering the activities of IL-4 and IL-5 as Th2 cytokines able to promote IgA transport and synthesis, respec-
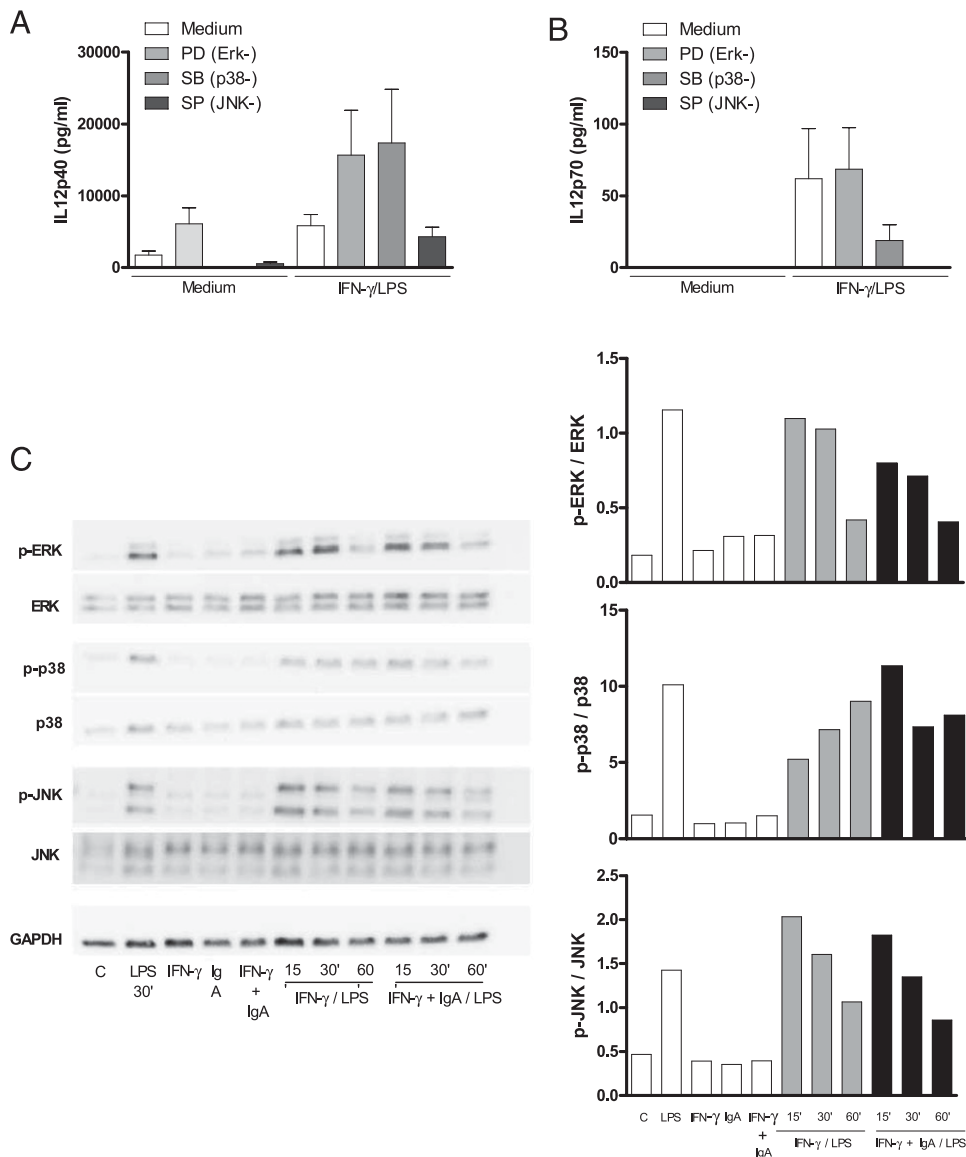
FIGURE 7. Effect of IgA on MAPK activation following IFN-γ priming and LPS stimulation. Effect of MAPK inhibitors (PD-98059, SB-203580, SP-600125 for ERK1/2, p38, JNK MAPKs, respectively) on p40 (**A**, *n* = 3) and IL-12p70 (**B**, *n* = 3) production by monocytes stimulated by IFN-γ and LPS, with or without IgA. (**C**) Effect of IgA on phosphorylation of ERK1/2, p38, and JNK MAPKs following IFN-γ priming (for 24 h) and LPS stimulation for 15, 30, or 60 min. Normalization was performed on the respective total (unphosphorylated) MAPKs and on GADPH, used as a loading control. *Right panels*, Quantification of each p-MAPK corrected for total MAPK. Data are representative of two independent experiments.

tively, a positive-feedback loop might operate between IgA regulation and Th2 mucosal immunity.

Our data show that IgA affects, through the regulation of myeloid cells, Th1-type immunity and the activation of STAT1 following LPS or IFN-γ activation. STAT1 is typically induced by (type 1 and 2) IFNs (27), whereas it is also induced by IL-12 itself, despite the fact that STAT4 represents the critical STAT for IL-12 signaling. Thus, this latter autocrine loop could underlie the observed inhibitory effect of IgA on total STAT1 levels. LPS, which directly triggers NF-κB activation, may induce STAT1 activation that is mediated by type I IFNs (IFN-α/β). Control of JAK/STAT-signaling pathway includes endogenous inhibitors [i.e., SOCS proteins consisting of proteins with a central SH2 domain and a C-terminal "SOCS" box (28)], which also regulate other cytokine and hormone receptors, such as epidermal growth factor receptor (29) and IL-10–driven inhibition of IFN-α– and IFN-γ–dependent genes (30). Therefore, it is tempting to speculate that IgA treat-

ment of monocytes and DCs interferes with IFN-γ signaling by recruiting endogenous inhibitors of STAT1.

Previous studies showed that ERK activation could mediate the suppressive effect of *Leishmania* phosphoglycans (31) or *Candida albicans* (32) on IL-12 expression, whereas this was not observed following FcγR (13) or FcαR ligation (this study). In line with previous studies in PBMCs (33) and monocytes/macrophages (5, 31), IL-12 production was independent of p38 MAPK activation in human monocyte–derived cells stimulated by IFN-γ and LPS; rather, it depended on JNK. Conversely, p38 MAPK inhibition upregulated p40 production in these studies, indicating a suppressor role on IL-12, which was also reported for JNK in human macrophages (34) and activated monocytes (35). Our data also show upregulation of IL-12 p40 upon ERK or p38 inhibition, whereas p38 or JNK targeting reduced the production IL-12p70. Nevertheless, IgA had no significant effect on MAPK activation that could underlie its inhibitory effect on IL-12.
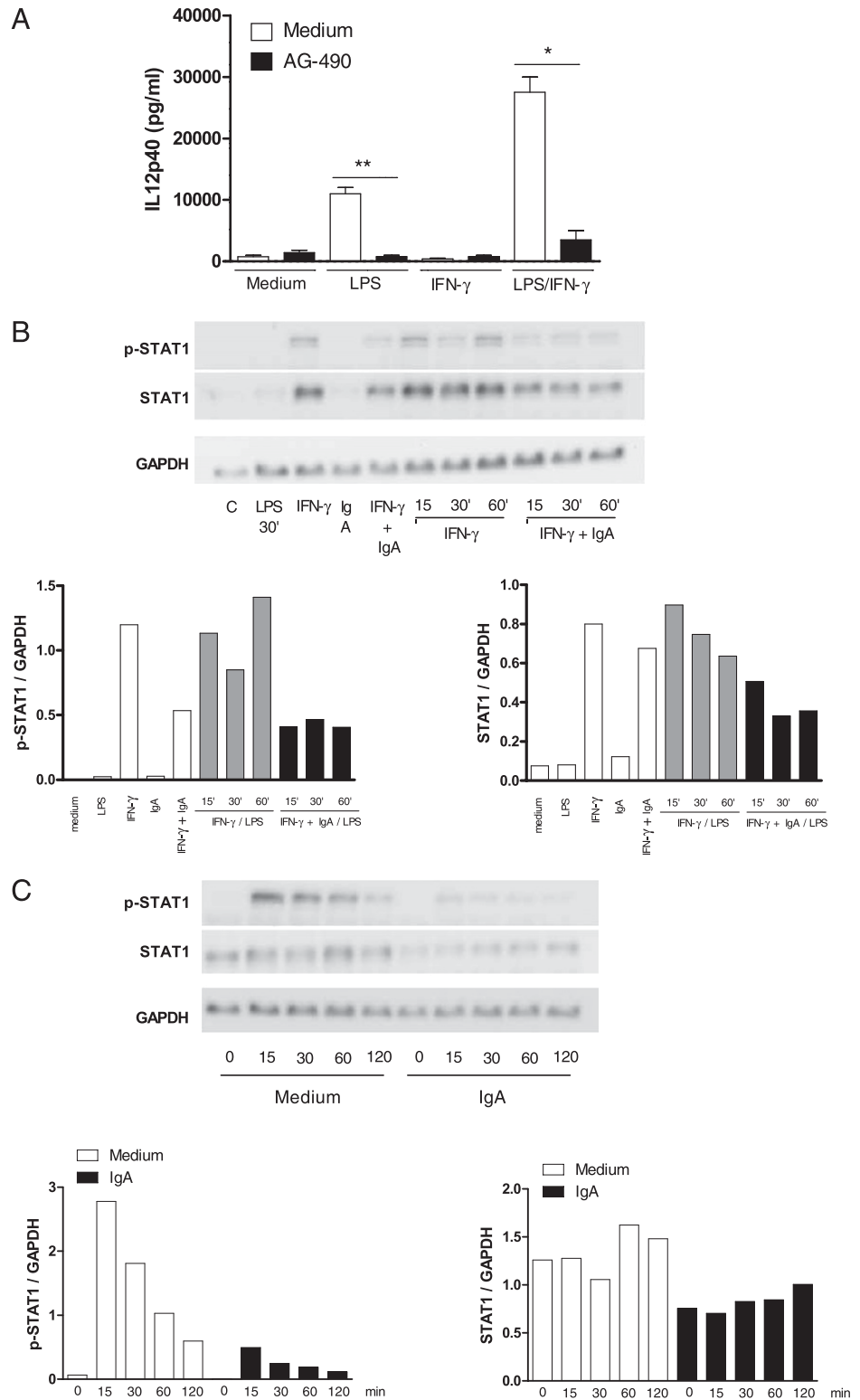
FIGURE 8. Effect of IgA on STAT1 activation following IFN-γ and/or LPS stimulation. (**A**) Effect of JAK2/STAT1 inhibitor (AG-490) on monocyte production of p40 following IFN-γ and/or LPS stimulation. Results depict mean ± SEM from two independent experiments. (**B**) Effect of IgA on STAT1 phosphorylation in monocytes primed by IFN-γ (for 24 h) and stimulated by LPS for 15, 30, or 60 min. Controls included IFN-γ, IFN-γ + IgA (for 24 h), and LPS alone (for 15 min). Normalization was performed on total (unphosphorylated) STAT1 and GAPDH. *Lower panels*, Quantification of p-STAT1 and total STAT1 corrected for GADPH. (**C**) Effect of IgA on STAT1 phosphorylation in monocytes activated by IFN-γ for 15–120 min. Normalization was performed on total (unphosphorylated) STAT1 and GAPDH. *Lower panels*, Quantification of p-STAT1 and total STAT1 corrected for GADPH. Data are representative of three independent experiments. $*p < 0.05$, $**p < 0.001$.

We previously showed that IgA may induce IL-10 expression in monocytes and MD-DCs (15), and IL-10 may also control IL-12 expression in an autocrine manner (16, 36), as observed following IgG ligation of FcγRs. However, autocrine IL-10 was not involved in the IgA-mediated inhibition of IL-12. Rather, we show that IL-12 suppression by IgA is recapitulated by FcαRI/CD89 cross-linking and extends to other signatures of type 1 activation in monocytes, such as cluster formation, release of CXCL10/IP10, and upregulated FcγRI/CD64 and HLA-DR and costimulatory

(CD80, CD86) molecules upon IFN-γ/LPS stimulation. Although it remains to be explored whether IgA specifically regulates M1 versus M2 differentiation, these data suggest that IgA could interfere with these proinflammatory pathways at the level of myeloid cell activation and that this occurs by suppressing STAT1 signaling.

IgA-mediated immunoregulation of IFN-γ responses may be relevant to T cell–mediated inflammatory diseases, particularly those associated with Th1-type immunopathology, such as sarcoidosis, Crohn's disease, multiple sclerosis, and experimental
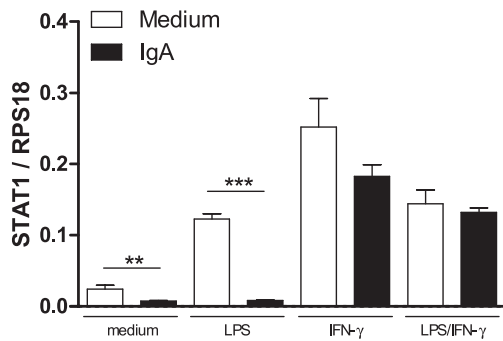
FIGURE 9. Effect of IgA on STAT1 expression following IFN-γ and/or LPS stimulation. RT-qPCR was carried out for STAT1 mRNA and corrected for RPS18. **$p < 0.01$, ***$p < 0.001$.

autoimmune encephalomyelitis. This might also be relevant to asthma, for which a dual role for IL-12 was demonstrated in a murine model (37). It was shown that, despite the fact that IL-12 counteracts Th2 responses during allergen sensitization, it can enhance, on further allergen exposure, the recruitment of CD4$^+$ T cells and granulocytes, as well as Th2 cytokines and chemokines; this unexpected proallergic role of IL-12 was not observed in IFN-γ–deleted mice. Also, upregulated STAT1 phosphorylation was detected in target tissue of inflammation in asthma (38), inflammatory bowel disease (39), and rheumatoid arthritis (40). In addition, our data showing IgA-mediated suppression of IFN-γ–driven CD64 expression and surface molecules involved in Ag presentation might suggest that IgA could represent a soluble factor that limits local activation of freshly recruited monocytes at mucosal tissue sites of ongoing inflammatory responses by suppressing IFN-γ–dependent activation of these proinflammatory pathways engaged for Ag presentation to, and type 1 activation of, T cells.

Interestingly, it was reported that selective IgA deficiency is associated with some Th1 diseases, such as Crohn's disease (41), and with upregulated IL-12 production (42), although the latter could not be localized to monocytes. It also remains possible that the association between autoimmunity and IgA deficiency relates to a common genetic susceptibility (43).

In conclusion, IL-12 plays essential roles in protective cellular immunity to intracellular pathogens by directing the development of Th1 cells, whereas uncontrolled inflammatory reactions may underlie several inflammatory diseases. We believe that the IgA/FcαRI-mediated regulatory pathway, identified in this in vitro study, may contribute to fine tuning of immune activation and prevention of Th1-mediated mucosal immunopathology by suppressing IFN-γ priming and STAT1 activation and, thereby, type 1 imprinting of monocytes and DCs. It remains to be investigated whether IgA is also able to turn off type 1 immune activation in vivo. This novel IgA pathway could participate to downregulate the immune potential to induce proinflammatory responses, particularly at mucosal surfaces that are continuously exposed to commensal and pathogenic microorganisms and Ags and where IgA is predominantly produced.

Disclosures
The authors have no financial conflicts of interest.

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