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Anti-inflammatory properties of human serum IgA: induction of IL-1 receptor antagonist and FcαR (CD89)-mediated down-regulation of tumour necrosis factor-alpha (TNF-α) and IL-6 in human monocytes

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SUMMARY

A deregulated expression and/or release of large amounts of inflammatory cytokines such as IL-1 and TNF-α accounts for most pathophysiological events in a variety of systemic inflammatory diseases, the effect being mediated by the interaction of these cytokines with their respective receptors. IL-1 receptor antagonist (IL-1Ra), mainly produced by monocytes/macrophages, is an inhibitor of IL-1 activity. The present study shows that human serum IgA induces significant IL-1Ra release in human peripheral blood mononuclear cells and adherent monocytes. IgA induced higher levels of IL-1Ra than *Haemophilus influenzae* type b (Hib) expressing lipopolysaccharide (LPS), purified LPS or phorbol myristate acetate (PMA), without induction of IL-1β release, and even inhibited LPS-induced IL-1β release. Induction of IL-1Ra by IgA could be detected both at the mRNA and protein levels in resting and activated monocytes. Ligation of FcαR with MoAb MY-43 or treatment with human serum IgA induced protein tyrosine phosphorylation in human monocytes, and herbimycin A, a specific inhibitor of protein tyrosine kinase activity, inhibited IgA-induced IL-1Ra production, suggesting that FcαR-mediated induction of tyrosine phosphorylation is required for the IgA-induced stimulation of IL-1Ra release. In addition, triggering of FcαR with MoAb specifically down-regulated TNF-α and IL-6 release in human monocytes activated with Hib. By the induction of IL-1Ra and down-regulation of the release of inflammatory cytokines such as IL-1β, TNF-α and IL-6, interaction of IgA with human monocytes may actively contribute to the regulation of the inflammatory response.

Keywords IgA FcαR IL-1Ra proinflammatory cytokines human monocytes

INTRODUCTION

IgA is the predominant immunoglobulin isotype in the mucosal surfaces of the respiratory, gastrointestinal and urogenital tracts, where it plays a central role in host defence against infection (reviewed in [1]). Secretory IgA is present in dimeric or polymeric form and is synthesized by local plasma cells before it is transported to the mucosal surfaces through epithelial transcytosis mediated by the polymeric immunoglobulin receptor, also known as the transmembrane form of the secretory component [1]. In contrast, IgA in serum is largely monomeric and derived from plasma cells in the bone marrow.

One of the main features of IgA antibodies appears to be their capacity to eliminate excess antigen of microbial, alimentary or inhaled origin without induction of inflammation, and to prevent the development of potentially harmful local and systemic inflammatory reactions induced by microbial products or immune complexes containing antibodies of other isotypes. IgA appears to have poor complement-activating ability when complexed to antigen [2,3], and competitively inhibits the reaction between antigen and antibodies of other isotypes with similar specificity, resulting in diminished complement activation [4]. A regulatory effect of IgA on phagocytic cells such as monocytes and neutrophils is brought about by the interaction of IgA with the FcαR (CD89) capable of binding monomeric IgA, secretory IgA and IgA immune complexes [5,6]. Interaction of IgA with FcαR has been reported to down-modulate directed locomotion [7–10], phagocytosis [11,12] and the generation of reactive oxygen radicals [13,14] in phagocytes. The aim of our investigation was the further clarification of the anti-inflammatory potential of IgA. Our results demonstrate that IgA interferes with the inflammatory response at different levels: by inhibition of the release of monocyte cytokines such as IL-1β, TNF-α and IL-6, as well as by interfering with cytokine–receptor interaction through induction of the IL-1 receptor antagonist (IL-1Ra), a naturally occurring inhibitor of IL-1 activity involved in the regulation of inflammation [15].

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MATERIALS AND METHODS

Human serum IgA preparation

Human serum IgA was prepared from a commercially available IgA-IgG preparation (IGABULIN; Immuno AG, Vienna, Austria) by ion exchange chromatography (kindly provided by Dr Y. Linnau, Immuno AG). The final IgA product contained >95% IgA, and IgG and IgM were below the detection limit as examined by single radial immunodiffusion. It was free of endotoxin contamination as examined by limulus amoebocyte lysate assay.

Induction of cytokine release

Human mononuclear cells (MNC) were isolated from heparinized peripheral blood of healthy adult blood donors as previously described [16]. For induction of cytokine release, 1×10^6 MNC/ml per well were incubated for 24 h in the presence of phorbol 12-myristate 13-acetate (PMA; Sigma Chemical Co., St Louis, MO), final concentration 100 ng/ml (0·162 μmol/l), purified lipopolysaccharide (LPS; prepared from *Escherichia coli* serotype 0111:B4 by phenol extraction; Sigma, no. L-2630, final concentration 10 ng/ml), heat-inactivated encapsulated *Haemophilus influenzae* type b (Hib; strain Eagan, kindly provided by Dr G. Zerlauth, Immuno AG; final concentration 1×10^6 bacteria/ml), purified human serum IgA, or medium alone (complete medium [16], containing 10% pooled, heat-inactivated (30 min, 56°C) fetal calf serum (FCS; HyClone Labs, Logan, UT)). Adherent human monocytes were prepared and cytokine release was stimulated after overnight culture in complete medium as previously described [16]. Mouse IgM MoAbs specific for CD14 (Mo2; Coulter Immunology, Hialeah, FL), CD11b (Mol; Coulter), or CD89 (FcαR, MY-43; Medarex, Inc., W. Lebanon, NH) were used in concentrations that were saturating in flow cytometry. To block tyrosine kinase activity, herbimycin A (Sigma; no. H-6649) was added to the cells during stimulation of cytokine release, and adherent monocytes were also pretreated with herbimycin A for 16 h at 37°C. IL-1β, IL-1Ra, TNF-α, IL-6, and granulocyte-macrophage colony-stimulating factor (GM-CSF) release were assessed in cell-free supernatants [16] using commercially available ELISA kits (IL-1β-EASIA, TNF-alpha-EASIA and IL-6-EASIA, Medgenix Diagnostics, Fleurus, Belgium; Quantikine Human GM-CSF Immunoassay and Quantikine Human Interleukin 1 Receptor Antagonist Immunoassay, R&D Systems, Minneapolis, MN). Results are expressed as ng/ml or as percentage of control relative to the cytokine release observed in monocytes stimulated with Hib or LPS alone.

Isolation of RNA and specific amplification of cDNA

After overnight culture in complete medium, adherent human monocytes were stimulated for 24 h with LPS (10 ng/ml) in the presence or absence of IgA (10 mg/ml). Unstimulated monocytes were cultured in medium alone. Total cellular RNA was then isolated according to the method of Chomczynski [17]. Equal amounts of total RNA were reverse-transcribed into cDNA by first and second strand synthesis employing avian myeloblastosis virus (AMV) reverse transcriptase (Boehringer Mannheim Biochemicals, Mannheim, Germany). The cDNA was directly amplified [18,19] on a BIO-MED Thermocycler 60 using AmpliTaq DNA polymerase (Perkin Elmer Cetus, Norwalk, CT) and oligonucleotide primer pairs specific for IL-1β (Clontech Labs, Palo Alto, CA), IL-1Ra (sense, GGAAATCTGCAGAGGCCTCCGCAGTC; antisense, GACCATGACGCCTTCGTCAGGCATATTGG) [20] and as internal control the S14 ribosomal protein (sense, GGCAGA-CCGAGATGAATCCTCA; anti-sense, CAG-GTCCAGGGGTCTTGGTCC) [21]. The amplification profile involved 30 cycles of denaturation at 95°C for 1 min, primer annealing at 60°C for 2 min and primer extension at 72°C for 3 min. Aliquots of polymerase chain reaction (PCR)-generated products were fractionated on 1·5% EtBr-agarose gels, validated by the predicted size, and blotted onto Hybond-N filter membranes according to the manufacturer's protocol (Amersham Int., Aylesbury, UK). The relative density of the signals obtained by Southern blot analysis of the PCR-generated products was determined by image-analysing densitometry (Pharmacia LKB Biotech., pdi Quantity One, Uppsala, Sweden). The densitometric reading of the indicated genes in a particular test was related to the corresponding densitometric reading for the internal control S14 ribosomal protein.

Immunoblot analysis of tyrosine phosphorylated monocyte proteins

After overnight culture in complete medium, adherent human monocytes were gently scraped off, washed, and resuspended at a concentration of 7×10^7 cells/ml in RPMI suppl. without FCS. Aliquots of 50 μl were transferred into 1·5-ml reaction tubes (Eppendorf-Hetheler-Hinz GmbH, Hamburg, Germany) and the cells were treated for 5 min at 37°C with human serum IgA (100 μg/ml) or different concentrations of the monoclonal FcαR antibody MY-43 diluted in PBS. Control cells were incubated for 5 min at 37°C in PBS alone. The cells were quickly washed with ice-cold PBS containing 1 mM Na_3VO_4, centrifuged for 10 s, and resuspended in 15 μl lysis buffer with inhibitors (25 mM Tris–HCl pH 7·5, 1% Nonidet P40, 150 mM NaCl, 0·1% NaN_3, 1 mM Na_3VO_4, 1 mM Na_2MoO_4, 2 mM phenylmethylsulfonyl fluoride, 10 μg/ml leupeptin, and 10 μg/ml aprotinin). The lysates were clarified by centrifugation for 10 min at 15 000 ***g*** and separated by SDS–PAGE under reducing conditions. Following electrophoresis, the proteins were transferred onto nitrocellulose sheets and probed with the phosphotyrosine-reactive MoAb 4G10 (Upstate Biotechnology Incorporated, Lake Placid, NY). After removal of excess antibody by vigorous washing with TBS plus 0·1% Tween 20, specific antibody binding was detected using the Enhanced Chemoluminescence Assay (Amersham).

Statistical analysis

Results are expressed as mean $\pm$ 1 s.e.m. of repeated experiments performed with cells from different healthy blood donors. For statistical evaluation of the difference between two study groups, the non-parametric Mann–Whitney U-test or the Wilcoxon matched-pairs signed-ranks test were employed. Statistical analysis of differences between more than two study groups was performed by calculating the non-parametric Kruskal–Wallis one-way ANOVA by ranks or the Newman–Keuls multiple comparisons test. $P < 0{\cdot}05$ was considered significant.

RESULTS

Human serum IgA induces IL-1Ra without stimulating IL-1β

The results presented in Fig. 1 show for the first time that human serum IgA induces significant IL-1Ra production in human MNC. Human serum IgA (10 mg/ml) induced significantly higher levels of IL-1Ra release compared with optimal concentrations of Hib, LPS or PMA, known to stimulate both IL-1β and IL-1Ra release

[22–24]. IgA stimulated IL-1Ra release without induction of IL-1β secretion (Fig. 1), and down-regulated IL-1β release in LPS-stimulated MNC (Fig. 2). In adherent human monocytes, IgA induced significant IL-1Ra release within a concentration range of 0·3–10 mg/ml in a dose-dependent manner, and stimulated IL-1Ra without activating TNF-α or IL-6 release (data not shown). Furthermore, IgA up-regulated Hib-induced IL-1Ra release in human monocytes (data not shown).

Induction of IL-1Ra by human serum IgA could also be demonstrated at the transcriptional level. In contrast to LPS, known to induce both IL-1β and IL-1Ra mRNA [23], IgA induced the expression of significant levels of IL-1Ra mRNA but only very low levels of IL-1β mRNA in adherent human monocytes, as examined by reverse transcriptase (RT)-PCR analysis (Fig. 3). Addition of IgA to LPS-stimulated monocytes led to an increase in levels of IL-1Ra mRNA detected, while levels of LPS-induced IL-1β mRNA remained unchanged (Fig. 3).

Protein tyrosine phosphorylation is an early event following ligation of monocyte FcαR by MoAb or IgA treatment and is required for IgA-mediated induction of IL-1Ra production

Interaction of IgA with human monocytes is mediated through FcαR (CD89) binding to the Fc portion of IgA [5,6]. Recently, it has been reported that in the promonocytic cell line U937 the non-receptor protein tyrosine kinase p72 syk is associated with FcαR, and that cross-linking of FcαR with the MoAb MY-43 stimulates induction of kinase activity of p72 syk and protein tyrosine phosphorylation of p72 syk as well as other cellular proteins involved in FcαR-mediated signal transduction [25]. The results presented show that also in human peripheral blood monocytes cross-linking of FcαR with IgM MoAb MY-43 is followed by the rapid induction of protein tyrosine phosphorylation. Furthermore, treatment with human serum IgA also induced protein tyrosine phosphorylation in human monocytes (Fig. 4). Bands of approximately 125, 116, 75, 48 and 42 kD molecular weight were detected in both IgA-treated and MY-43-treated monocytes by anti-phosphotyrosine immunoblotting (Fig. 4). Induction of tyrosine phosphorylation was specific for FcαR triggering, as protein tyrosine phosphorylation was not increased over background in control monocytes treated with either isotype-matched MoAb against CR3 (Mo1) or up to 1 μg/ml of LPS (data not shown).

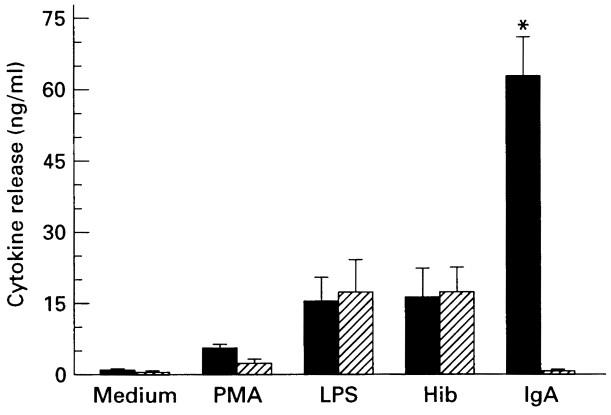

Fig. 1. Human serum IgA induces significant IL-1Ra release in human peripheral blood mononuclear cells (PBMC) without stimulation of IL-1β release. PBMC were incubated for 24 h in the presence of phorbol myristate acetate (PMA; 100 ng/ml), lipopolysaccharide (LPS; 10 ng/ml), heat-inactivated *Haemophilus influenzae* type b (Hib; 1×10^6 bacteria/ml), human serum IgA (10 mg/ml) or medium alone. IL-1Ra (■) and IL-1β (▨) release were measured by ELISA. Results of three experiments are given as mean ± s.e.m. *Statistically significant difference compared with IL-1Ra production in control cells or cells stimulated with PMA, LPS or Hib ($P < 0{\cdot}05$, Newman–Keuls multiple comparisons test).

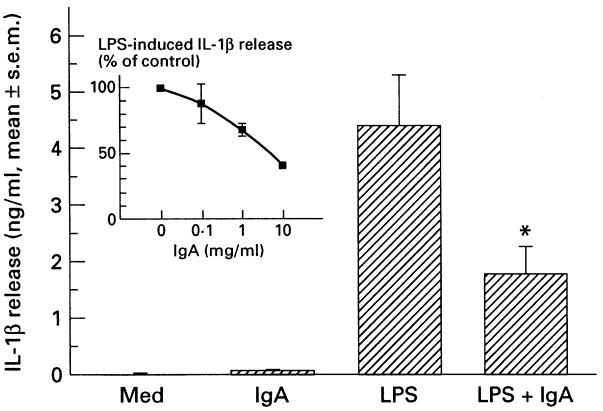

Fig. 2. Human serum IgA inhibits lipopolysaccharide (LPS)-induced IL-1β release in human peripheral blood mononuclear cells. MNC (1×10^6 cells/ml per well) were stimulated for 24 h with LPS (10 ng/ml) in the presence or absence of human serum IgA (10 mg/ml). Control cultures included cells incubated in the presence of IgA or medium alone. IL-1β release (▨) was measured by ELISA, and results are given as ng/ml (mean ± s.e.m. of three experiments). The inset shows dose-dependent inhibition of LPS-induced IL-1β release by IgA as percentage of control IL-1β release (■, mean ± s.e.m. of the three experiments). *Statistically significant inhibition of IL-1β release ($P = 0{\cdot}022$, Mann–Whitney *U*-test; $P = 0{\cdot}002$, Kruskal–Wallis one-way ANOVA by ranks).

The results presented in Fig. 5 show the important role of protein tyrosine phosphorylation in IgA-mediated activation of monocyte functions, such as IgA-mediated induction of IL-1Ra release. Herbimycin A (1μg/ml), a specific inhibitor of protein tyrosine kinase activity [26,27], significantly inhibited the IgA-mediated induction of IL-1Ra release in human MNC by 71 ± 3% (mean ± s.e.m. of three experiments, Fig. 5). The inhibitory effect of herbimycin A was specific for IgA-mediated IL-1Ra release, as PMA-induced IL-1Ra production, known to be stimulated by direct activation of protein kinase C, a serine/threonine-specific protein kinase, was unaffected (Fig. 5). When herbimycin A was present before and during IgA treatment, herbimycin A at a concentration of 0·1 μg/ml completely inhibited induction of IL-1Ra release in human monocytes (data not shown).

Triggering of FcαR (CD89) modulates TNF-α and IL-6 release in human monocytes

The results presented in Fig. 6 confirm and extend previous studies [16] by showing that the IgA-mediated down-regulation of TNF-α and IL-6 release in Hib-stimulated human monocytes is due to FcαR triggering. Hib-induced TNF-α and IL-6 release could be inhibited by triggering of FcαR (CD89) with human serum IgA as well as with an IgM MoAb, MY-43 [28], specific for the ligand binding site of FcαR (Fig. 6). Comparable to IgA, FcαR MoAb had no effect on Hib-induced GM-CSF release (Fig. 6). Other mouse MoAbs of the same isotype (IgM), but different specificity (CD14 or CD11b), had no effect on Hib-induced monocyte cytokine

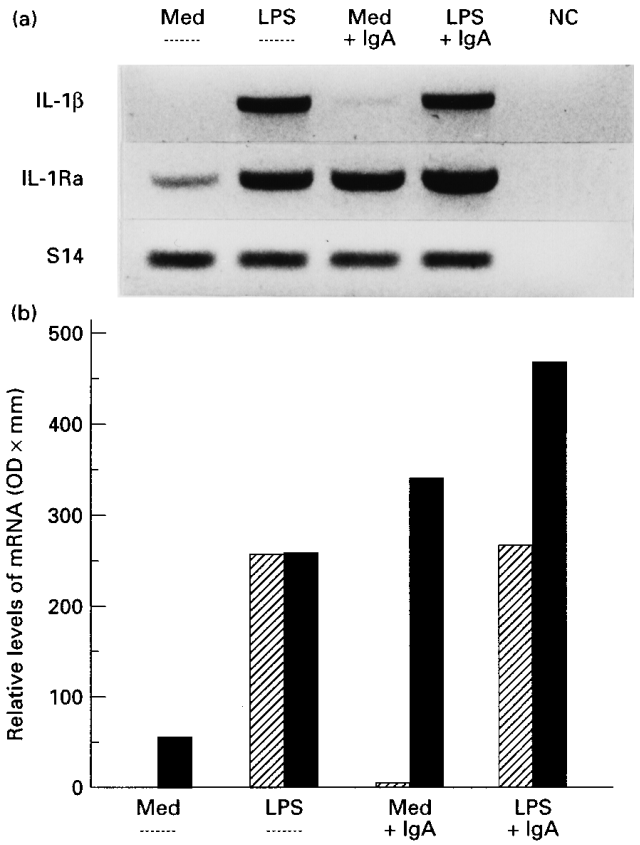

Fig. 3. IgA induces expression of IL-1Ra mRNA in human monocytes. (a) Southern blot analysis of amplified cDNA from adherent human monocytes. RNA was isolated from unstimulated (Med) and lipopolysaccharide (LPS)-stimulated human monocytes (LPS, 10 ng/ml) cultured in the presence of IgA (10 mg/ml) or medium alone for 24 h. RNA was reverse-transcribed and amplified with the indicated primer pairs (IL-1β, IL-1Ra and S14). Reverse transcriptase-polymerase chain reaction (RT-PCR) assayed without cDNA template served as a negative control (NC). (b) Densitometric quantification of Southern blot analysis of amplified cDNA encoding IL-1β and IL-1Ra. Southern blot analysis of the PCR-generated products depicted in (a) was quantified by image-analysing densitometry. The densitometric reading for IL-1β (▨) and IL-1Ra (■) was related to the corresponding reading for the internal control, ribosomal protein S14. Relative levels of mRNA are presented as optical density × mm (OD × mm).

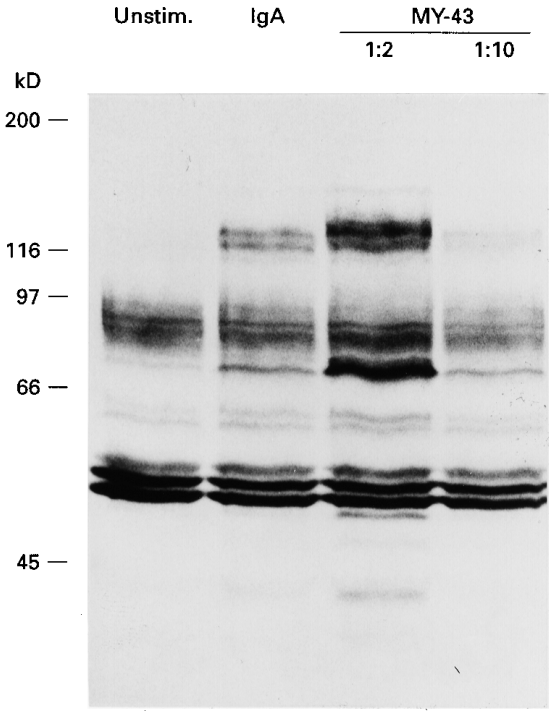

Fig. 4. Human serum IgA and the FcαR MoAb MY-43 induce tyrosine phosphorylation of cellular proteins in human monocytes. Human monocytes purified by adherence to plastic surfaces were gently scraped off, stored on ice for 1 h ($3{\cdot}5 \times 10^6$ cells/tube), incubated for 2 min at 37°C and then treated for 5 min at 37°C with human serum IgA (100 μg/ml) or the FcαR MoAb MY-43 diluted in PBS. Control cells were left untreated at 37°C for 5 min. The cells were quickly washed once with ice-cold PBS containing 1 mM Na_3VO_4 and afterwards lysed in NP-40 lysis buffer for 20 min on ice. Soluble cellular proteins were separated on 8% polyacrylamide gels under reducing conditions, transferred onto nitrocellulose sheets and probed with phosphotyrosine-reactive MoAb 4G10. Molecular weights in kD are indicated on the left.

release (Fig. 6). The inhibitory effect of FcαR MoAb on monocyte cytokine release was dose-dependent over approximately one log-range, as could be shown for Hib-induced TNF-α release (data not shown).

DISCUSSION

The present results clearly show that human serum IgA plays an active role in the regulation of the inflammatory reponse independent of its antibody activity. IgA has the capacity to induce the production of high levels of IL-1Ra, a naturally occurring inhibitor of IL-1 activity previously shown to be produced by monocytes/macrophages in response to FcγR triggering or LPS [22,23,29,30]. This regulatory molecule binds to IL-1 receptors without exerting agonist activity, thereby blocking the binding of IL-1 to its receptor and inhibiting IL-1 activity.

Induction of IL-1Ra by IgA could be detected both at the mRNA and protein levels in resting and activated monocytes. IgA stimulated significantly higher levels of IL-1Ra production than LPS-expressing Hib, LPS or PMA employed at an optimal concentration, and most remarkably induced IL-1Ra production without stimulating the release of proinflammatory cytokines such as IL-1β, TNF-α or IL-6. On a dose–response basis, human serum IgA was more efficient in inducing IL-1Ra release than monomeric soluble human IgG examined in parallel, which is known to be a potent stimulus for IL-1Ra production [31–33], although semi-quantitative determination with flow cytometry demonstrated that both immunoglobulin preparations bind comparably to human monocytes (data not shown). In addition, human serum IgA stimulated IL-1Ra release even when endotoxin activity was blocked by polymyxin B (100 μg/ml, data not shown), indicating that IgA and endotoxin stimulate IL-1Ra production through different mechanisms.

A previous study showed that IgA down-modulates the release of proinflammatory cytokines such as TNF-α and IL-6 in activated human monocytes [16]. The present results extend these findings by showing that IgA also down-regulates LPS-induced IL-1β

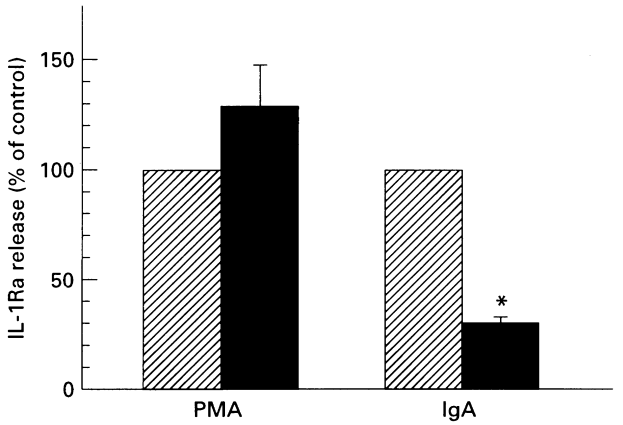

Fig. 5. Herbimycin A inhibits IgA-induced IL-1Ra release. Human peripheral blood MNC were incubated for 24 h in complete medium containing phorbol myristate acetate (PMA; 100 ng/ml) or IgA (3 mg/ml) in the presence (■) or absence (control, ▨) of herbimycin A (1 μg/ml). IL-1Ra release was then determined by ELISA, and results are given as percentage of control, i.e. relative to the IL-1Ra release observed in cells stimulated in the absence of herbimycin A (PMA, $5{\cdot}1 \pm 0{\cdot}9$ ng/ml; IgA, $98{\cdot}8 \pm 25{\cdot}0$ ng/ml). Cells cultured in medium alone released $0{\cdot}4 \pm 0{\cdot}3$ ng/ml of IL-1Ra, and cells cultured in medium containing only herbimycin A released $0{\cdot}2 \pm 0{\cdot}1$ ng/ml of IL-1Ra (mean ± s.e.m. of three experiments). *Statistically significant inhibition compared with cells stimulated in the absence of herbimycin A (IgA-induced IL-1Ra release in the presence of herbimycin A, $30{\cdot}6 \pm 9{\cdot}8$ ng/ml; in the absence of herbimycin A, $98{\cdot}8 \pm 25{\cdot}0$ ng/ml; mean ± s.e.m. of three experiments, $P = 0{\cdot}024$, Mann–Whitney *U*-test).

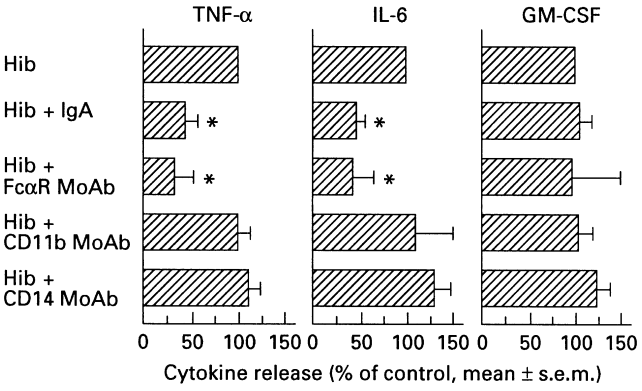

Fig. 6. Human serum IgA and triggering of FcαR with MoAb down-regulate *Haemophilus influenzae* type b (Hib)-induced TNF-α and IL-6 release in human monocytes, while granulocyte-macrophage colony-stimulating factor (GM-CSF) release is unaffected. Adherent human monocytes were stimulated for 24 h with Hib in the presence of human serum IgA (3 mg/ml), MoAbs specific for FcαR (MY-43), CD11b (Mol) or CD14 (Mo2), or medium alone. TNF-α, IL-6 and GM-CSF release were measured by ELISA; results are given as percentage of control cytokine release (mean ± s.e.m. of three to six experiments), relative to the cytokine release in control monocytes cultured in the presence of Hib alone (cytokine release in these control monocytes, ng/ml, mean ± s.e.m. of six experiments: TNF-α, $11{\cdot}5 \pm 3{\cdot}3$; IL-6, $4{\cdot}6 \pm 1{\cdot}0$; GM-CSF, $0{\cdot}4 \pm 0{\cdot}9$). Monocytes cultured in the presence of medium alone released $0{\cdot}037 \pm 0{\cdot}017$ ng/ml of TNF-α, $0{\cdot}041 \pm 0{\cdot}029$ ng/ml of IL-6, and $0{\cdot}016 \pm 0{\cdot}011$ ng/ml of GM-CSF (mean ± s.e.m., $n = 6$). In the presence of IgA alone, cytokine release was $0{\cdot}232 \pm 0{\cdot}081$ ng/ml for TNF-α, $0{\cdot}082 \pm 0{\cdot}045$ ng/ml for IL-6, and $0{\cdot}008 \pm 0{\cdot}005$ ng/ml for GM-CSF ($n = 5$). Mean cytokine relase by monocytes cultured in the presence of MoAbs CD14, CD11b or MY-43 alone was <0·065 ng/ml for TNF-α, <0·090 ng/ml for IL-6, and <0·020 ng/ml for GM-CSF. *Statistically significant difference compared with control cytokine release in monocytes stimulated with Hib alone ($P = 0{\cdot}0295$, Wilcoxon matched-pairs signed-ranks test; Kruskal–Wallis one-way ANOVA by ranks of the percentages of control cytokine release: TNF-α, $P = 0{\cdot}004$; IL-6, $P = 0{\cdot}013$, GM-CSF, $P = 0{\cdot}523$).

release, thus indicating that IgA has the capacity to down-regulate IL-1 activity at two levels, by induction of IL-1Ra and by down-regulating IL-1β induction and/or release. Various mechanisms are feasible whereby human serum IgA prepared from a large plasma donor pool could down-regulate the activity of proinflammatory cytokines. Antibody activities present in pooled immunoglobulin preparations due to the large antibody repertoire of the donor population (e.g. antibodies to alloantigens or other cell surface molecules [34–37], antibodies against bacterial components such as LPS [38], cytokine-binding antibodies [39]) could down-regulate cytokine production by inhibition of ligand–receptor interaction. However, previous findings suggested that the IgA-mediated down-regulation of cytokine release in Hib-activated human monocytes is isotype-specific, since a pooled IgG preparation was not inhibitory [16]. Isotype-specific interaction of IgA with human monocytes is mediated by FcαR (CD89), capable of binding monomeric, dimeric and polymeric IgA [5,6]. The present findings provide additional evidence that the IgA-mediated down-regulation of cytokine release in human monocytes is indeed isotype-related and involves triggering of the monocyte FcαR. Comparable to the previously described down-modulation of TNF-α and IL-6 by IgA [16], a MoAb against the ligand binding site of the phagocyte FcαR [28] specifically down-regulated Hib-induced TNF-α and IL-6 release in human monocytes, while GM-CSF release was unaffected.

The concept that isotype-specific interaction of IgA with its receptor on phagocytic cells has a regulatory effect on the inflammatory response is further supported by our finding that FcαR MoAb was capable of activating IL-1Ra production, while CD14- or CD11b-specific MoAbs had no effect (data not shown). Recently it has been reported that signal transduction following FcαR cross-linking involves induction of protein tyrosine kinase (PTK) activity. In the promonocytic cell line U937 FcαR has been shown to be associated with the non-receptor PTK p72 syk, and triggering of FcαR stimulated tyrosine phosphorylation and activation of p72 syk [25]. Our results extend these findings by showing that both FcαR MoAb and human serum IgA induced protein tyrosine phosphorylation in human peripheral blood monocytes with a comparable pattern of protein bands detected by immunoblotting, indicating that tyrosine phosphorylation of proteins involved in signal transduction might be an important step in FcαR-mediated monocyte activation. The importance of protein tyrosine phosphorylation for FcαR signal transduction is ascertained by the finding that herbimycin A, a specific PTK antagonist, inhibited IgA-mediated but not PMA-mediated IL-1Ra release, thus indicating that FcαR-mediated protein tyrosine phosphorylation is essential for induction of IL-1Ra release by IgA. However, a series of additional experiments will be required to determine whether monomeric IgA or IgA-dimers and/or polymers are equally capable of triggering IL-1Ra release.

The present findings suggest an important role for IgA as a physiologic regulator of the inflammatory response. High levels of proinflammatory cytokines such as TNF-α, IL-1, IL-6 and IL-8 have been described in inflammatory diseases such as bronchial asthma, inflammatory bowel syndrome and neonatal necrotizing

enterocolitis [40–44]. To some extent, physiological counterregulation of the activity of proinflammatory cytokines is provided by the concomitant induction of regulatory molecules such as IL-1Ra by microbial components (e.g. LPS) or FcγR cross-linking by IgG-containing immune complexes [22,23,29,30]. However, the regulatory potential of cytokine inhibitors produced in response to proinflammatory stimuli could be insufficient in an overwhelming acute or chronic inflammatory response associated with the release of high levels of proinflammatory cytokines [45–47]. By controlling the activity of proinflammatory cytokines at two distinct levels, i.e. at the level of cytokine induction/release and through down-regulation of cytokine activity by induction of a regulatory cytokine antagonist, IgA could interfere with positive feedback loops leading to the production of high levels of inflammatory cytokines, and would thus significantly contribute to the maintenance and restoration of the physiological balance between proinflammatory and anti-inflammatory mechanisms.

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