Immunoglobulin D Enhances Interleukin-6 Release from the KU812 Human Prebasophil Cell Line

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Abstract. Despite the role of secreted immunoglobulin D (IgD) remains still largely unknown, previous studies have suggested that secreted IgD could induce basophils degranulation in some allergic asthma patients. In the present study we have searched direct evidence of the action of IgD on KU812 cells, generally classified as an immature basophilic cell line. We analyzed by flow cytometry the capacity of IgD, purified from IgD myeloma sera, to bind KU812 cells. Biotinylated monomeric IgD (mIgD) and biotinylated oligomeric IgD (oIgD) could bind KU812 cells. Blocking experiments with others immunoglobulin isotypes showed that KU812 cells expressed an unspecific receptor for IgD. However, oIgD but not mIgD enhances the release of interleukin-6 (IL-6) from KU812 cells. On the other hand, mIgD and oIgD failed to induce histamine release from KU812 cells or from cord blood derived basophils. Since IL-6 is known to induce basophil differentiation, we proposed that IgD could be implicated in allergic disorders by stimulating IL-6 release by prebasophil cells, then IL-6 could further induce an autocrine maturation of the cells.

Key words: Immunoglobulin D — Prebasophil — Interleukin-6 — Flow cytometry — Histamine

Introduction

Since the discovery of immunoglobulin D (IgD) in 1965, studies have mainly been focused on membrane IgD which is a major component of the B cell antigen receptor (BCR). In contrast, the action and significance of secreted IgD remain still largely

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unknown (Rowe et al. 1973; Nitschke et al. 1993; Roes and Rajewsky 1993). IgD is a quantitatively minor component of serum immunoglobulin and constitutes only 0.2 % of all immunoglobulins. In contrast to IgG, IgA and IgM a trimodal distribution of IgD levels in normal human sera has been observed (Dunnette et al. 1977). IgD has a half-life of 2.8 days and is the most rapidly cleared antibody of the five classes. IgD has been found to be increased in a number of diseases such as IgD myeloma, tuberculosis, chronic obstructive pulmonary diseases, Hodgkin's disease, autoimmune disorders, hyperIgE and hyperIgD syndromes.

IgD receptors are detected on human T and "non-T" lymphocytes despite the absence of further molecular characterization (Sjörberg 1980). T cells with receptors for IgD, also called Tδ cells, were described in human peripheral blood and in murine peripheral lymphoid organs. *In vitro* exposure to oIgD but not to mIgD causes a rapid upregulation of the expression of IgD-receptor (Coico et al. 1985, 1990).

The results from several studies raised the question of a role for secreted IgD in allergic disorders. IgD in the serum has been found to be increased in atopic disease, allergic asthma and allergic bronchopulmonary aspergillosis (Kohler and Farr 1967; Luster et al. 1976; Kikindjanin 1981). So far, allergen-specific IgD antibodies have been reported against penicillin (Gleich and Stankievic 1969), house dust mite, cat dander and pollen in allergic patients. In contrast to total IgE and IgD levels in serum, significant correlations were observed between allergen specific-IgD and allergen specific-IgE (Zhang et al. 1994). On the other hand, anti-IgD could induce basophils degranulation in some allergic asthma patients indicating that probably IgD receptors could bind IgD on the basophil surface, a well known phenomenon induced by IgE on basophils and mediated by high-affinity Fcε-receptors (FcεRI) (Yi-Ping 1980; Peng et al. 1991). mIgD has been previously shown *in vitro* to enhance the release of various proinflammatory cytokines from human peripheral blood mononuclear cells (PBMC), including IL-6 (Drenth et al. 1996). IL-6 is spontaneously expressed in the human prebasophilic cell line KU812 cells, and it has been proposed to be involved in terminal differentiation towards basophil by an autocrine loop (Navarro et al. 1990; Nilsson et al. 1994). To our knowledge the action of IgD on IL-6 release by cell lineages as basophils has not been investigated. Independently it was demonstrated in mice with a homozygous null mutation of the Cε gene that hypersensitivity reactions could be induced by ovalbumin. This suggested that IgE-independent pathways may be involved in this phenomenon (Oettgen et al. 1994).

The present study investigated the binding of IgD to the immature basophilic cell line KU812 by flow cytometry and the potential triggering and release of mediators from basophilic cells. The results indicate that IgD bind to KU812 cells in an unspecific and unsaturable fashion. IgD could enhance IL-6 release by KU812 cells, but could not induce IL-6 and histamine production by basophilic cells derived from human cord blood.

Materials and Methods

Basophilic cells

KU812, a human cell line established from a patient with basophilic leukemia in blast crisis (Kishi 1985) was grown in RPMI-1640 medium containing 10% heat inactivated fetal calf serum, 2 mmol/l L-glutamine, 100 μg/ml penicillin and 100 μg/ml streptomycin. Basophils were derived from hematopoietic cells originating from cord blood as previously described (Arock et al. 1993).

Reagents

IgD were purified from human IgD myeloma sera by ammonium sulfate precipitation followed by affinity-purification using immobilized jacalin (which binds IgD and IgA1) and then by a negative affinity-purification to eliminate IgA. 5 mmol/l ε-aminocaproic acid and 1 mmol/l phenylmethylsulfonyl fluoride were added to the serum and all buffers in order to inhibit IgD proteolysis. Immunoglobulins were precipitated with ammonium sulfate at 40% saturation during 30 min at 4℃ and the preparation was kept overnight at 4℃. After centrifugation, the precipitate was suspended in phosphate-buffered saline (PBS), pH 7.2 and dialyzed against PBS. The preparation was incubated with jacalin cross-linked to beaded agarose (Pierce, Rockford, USA) for 90 min at 25℃ in PBS. Immobilized jacalin was washed with 1 liter PBS to remove non specific binding. IgD and IgA1 were further eluted with 50 ml 0.8 mol/l D-galactose, and dialyzed against 10 mmol/l Tris, pH 8.0. This fraction was incubated with sheep-anti-human-IgA (Silenus, Hawthorn, Australia) coupled sepharose 4B (Pharmacia, Uppsala, Sweden) for 2 h at 25℃ in order to eliminate IgA1. After centrifugation, the supernatant was filtered (Minisart, 0.2 μm, Sartorius, Göttingen, Germany) and concentrated (Centricon Plus-20, Millipore, Bedford, USA). No IgG, IgE, IgM, IgA or serum protein can be detected by laser immunonephelemetry and immunodiffusion assays in this preparation. The analysis of the IgD preparation by SDS-PAGE on a 4–20% polyacrylamide gel showed a major band of Mr 186,000 indicating that the protein had not undergone degradation during purification.

IgE was purified from a human myeloma sera by the combination of precipitation with ammonium sulfate at 55% saturation, gel filtration chromatography (ACA 34, IBF, Villeneuve la Garenne, France) and anion exchange HPLC chromatography (MonoQ HR5/5, Pharmacia, Uppsala, Sweden). Purified IgA1, IgG1 and IgM were generously provided by J.-L. Preud'homme (Poitiers, France).

Polymerization of IgD was carried out as previously described (Daugharty et al. 1969). oIgD and mIgD were biotinylated using a method described by Coico et al. (1990).

Cell stimulations

One million cells were stimulated in 1 ml RPMI medium at 37℃ during 1 h for histamine or 18 h for IL-6 measurement in supernatants. Cells were incubated

in medium alone or with 40 nmol/l phorbol 12-myristate 13-acetate (PMA) and 480 nmol/l calcium ionophore A23187, 50 μg/ml mIgD, 50 μg/ml oIgD, 50 μg/ml mIgD and 10 μg/ml anti-IgD (Dako, Glostrup, Denmark), 1 μg/ml IgE and 5 μg/ml anti-IgE (Southern Biotechnology, Birmingham, USA), 5 μg/ml anti-IgE, 10 μg/ml anti-IgD. After centrifugation the supernatants were harvested and frozen until use. Cell-associated histamine and histamine release were determined by a specific immunoassay kit (Immunotech, Marseille, France). For the measurement of intracellular histamine, the cells were suspended in water and were lysed by freezing-and-thawing. IL-6 was assayed by enzyme linked immunosorbent assay as previously described (Dore et al. 1997).

Flow cytometric analysis was performed with a FACScan (Becton-Dickinson, USA), and 10,000 cells were analyzed in each experimental condition. Five hundred thousandcells were incubated (as in Coico et al. 1990) with 50 μg/100 μl biotinylated IgD (Biot-IgD) in PBS containing 1% (w/v) bovine serum albumin (BSA). The cells were washed twice, and further incubated for 30 min with 40 μg/ml streptavidin-FITC. Controls cells were stained with streptavidin-FITC only. Surface expression of FcεRI was assessed using 10 μg/ml mouse-anti-human FcεRI receptor antibodies (kindly supplied by Dr. Benhamou, Paris, France).

Results and Discussion

In the present work, we investigated the action of IgD on cells of the KU812 line, sharing with normal basophils the expression of a number of cell surface markers, including the FcεRI, and generally accepted as an immature basophilic cell line. To study IgD binding to KU812 cells, we used Biot-IgD at concentrations previously used for characterization of IgD receptor on Tδ cells (Coico et al. 1990). We showed direct mIgD and oIgD binding to KU812 cells and as previously reported FcεRI expression (Fig. 1). Up to 5 mg/ml IgD failed to reach any saturable binding of IgD on KU812 cells (data not shown). IL-2, IL-4, IFN-γ, PMA, oIgD, previously reported to upregulate IgD-receptor expression on Tδ cells (Amin et al. 1988), had no effect on this expression. To investigate the specificity of IgD binding to KU812 cells, blocking experiments (Magnusson et al. 1995) were performed with 50 μg/100 μl IgG1, IgM, IgE and IgA1 purified myeloma immunoglobulins. As shown in Fig. 2, all of these immunoglobulin preparations could block the binding of Biot-IgD. Biotinylated IgE (Biot-IgE) also bind to KU812 cells, according to previous reports (Magnusson et al. 1995). In contrast to IgD, IgE binding can be blocked only by IgE itself indicating that the classical high-affinity FcεRI receptor is involved in such binding. Altogether, these data strongly indicated that KU812 cells do not express a specific receptor for IgD. Interestingly, it has recently been shown that human T-cell IgD-receptor is a lectin and binds IgA1 as well as IgD (Swenson et al. 1998).

Histamine content in the KU812 cells was quite low and KU812 failed to respond to the PMA/calcium ionophore A23187 or to IgE/anti-IgE, known to induce histamine release in basophils (Arock et al. 1993). Previous works had suggested

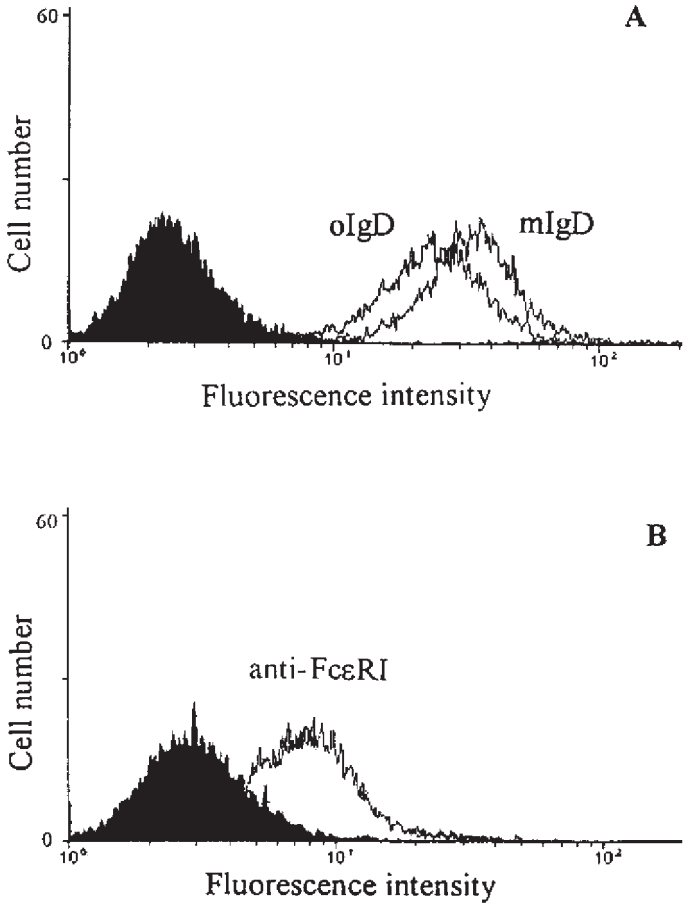

Figure 1. Flow cytometry analysis of the binding of mIgD and oIgD and the expression of FcεRI on KU812 cells. Five hundred thousand cells were used in each condition. Control staining is shown in shaded curve. **A.** Result of a representative experiment from 5 independent experiments. **B.** Irrelevant isotype-matched mAb labeling is similar to the control. Result of a representative experiment from 3 independent experiments.

that KU812 cells are immature with respect to the synthesis of histamine or enzymes involved in histamine release (Fischkoff et al. 1987). We also investigated the action of IgD on histamine release using basophils obtained in long-term culture of cord blood derived hematopoietic cells (not shown). Upon PMA/calcium ionophore A23187 and IgE/anti-IgE activation, 40 and 19% of histamine cell content were released respectively, *versus* 4% for the control, but mIgD, oIgD or mIgD/anti-IgD failed to induce significant release (4.6 and 5.5% of cell content, respectively).

IL-6 and IL-6 receptors are spontaneously expressed in the human KU812 cell

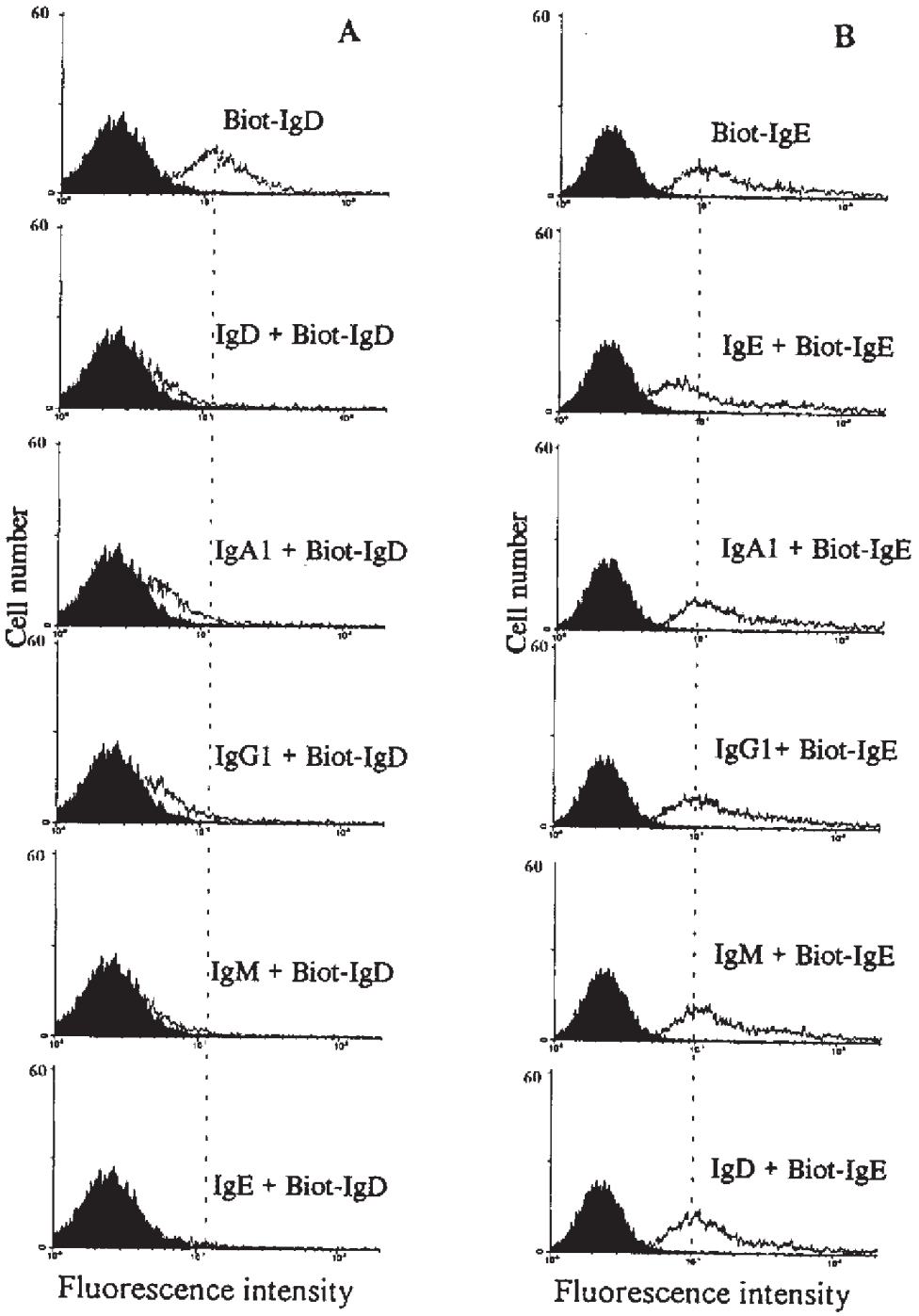
A
Biot-IgD
IgD + Biot-IgD
IgA1 + Biot-IgD
IgG1 + Biot-IgD
IgM + Biot-IgD
IgE + Biot-IgD
Cell number
Fluorescence intensity
B
Biot-IgE
IgE + Biot-IgE
IgA1 + Biot-IgE
IgG1+ Biot-IgE
IgM + Biot-IgE
IgD + Biot-IgE
Cell number
Fluorescence intensity

Table 1. IL-6 release by exposure of KU812 cells to IgD

Culture conditions	IL-6 release
control	222 ± 20[a]
mIgD	230 ± 14
oIgD	636 ± 50
mIgD/anti-IgD	171 ± 9

[a]IL-6 levels (pg/ml ± S.E.) in KU812 cells supernatants following incubation in the presence of 50 μg/ml mIgD, 50 μg/ml oIgD or 50 μg/ml mIgD/5 μg/ml anti-IgD for 18 h. Mean from five experiments. $p < 0.005$ when comparing KU812 cells treated with oIgD with untreated cells.

line (Navarro et al. 1990), and IL-6 has been proposed to be involved in terminal differentiation towards basophilic lineage by an autocrine loop (Nilsson et al. 1994). Since mIgD *in vitro* can induce IL-6 release from PBMC (Drenth et al. 1996), we wondered if IgD would be able to induce IL-6 release from KU812 cells. Significant levels of IL-6 (154 pg/10^6 cells/24 h) could be detected in the supernatants of KU812 cells (Krüger-Krasagakes et al. 1996). As shown in Table 1, oIgD but not mIgD or mIgD/anti-IgD can enhance the spontaneous release of IL-6. IgE/anti-IgE, which induce significant histamine release from basophils, failed to induce IL-6 release from KU812 cells. Likewise, anti-IgD and anti-IgE could not induce IL-6 release from these cells (data not shown). These data suggested that cross-linking of the putative IgD receptor on KU812 cells by an oligomeric form of IgD was a prerequisite for induction of IL-6 release. Interestingly, an IL-6 production by human neutrophils after Fcγ-receptor cross-linking has recently been shown (Ericson et al. 1998). Using the same stimuli, no IL-6 was detected in the supernatants from basophils originating from cord blood (below 8 pg/ml). We suggested that *in vivo*, increased IgD levels in the serum of atopic or allergic asthma patients could combine to allergen to create complexes. This putative oligomeric IgD could serve as a continuous stimulus for IL-6 production by immature basophils.

Whereas two precedent studies hypothesized IgD induced histamine release (Yi-Ping 1980; Peng et al. 1991), we showed that IgD could not induce a significant histamine release from basophils. Immature basophil cells are able to bind IgD, but these binding sites appear to be unspecific of IgD since IgG, IgA, IgE and IgM are

Figure 2. Flow cytometry analysis of the blocking activity of purified IgD, IgA1, IgG1, IgM and IgE myeloma proteins on subsequent Biot-IgD binding to KU812 cells. Five hundred thousand cells were preincubated with 50 μg/100 μl of the myeloma proteins to be tested for 1 h and washed 3 times with PBS-1% BSA. **A.** Thereafter, 50 μg/100 μl Biot-mIgD was added for 30 min and the cells were treated for flow cytometry analysis. Control staining is shown in shaded curve. Result of a representative experiment from 3 independent experiments. **B.** A control experiment was made on Biot-IgE binding to KU812 cells.

able to block the binding of IgD. Nevertheless, oIgD enhances the release of IL-6 from these cells. These results suggested that IgD could be implicated in allergic disorders by its stimulatory activity on the autocrine loop of IL-6 on basophil differentiation.

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