The CD19 Molecule is Crucial for MID-Dependent Activation of Tonsillar B Cells from Children

R. Hadzic*, A. Forsgren*, L.-O. Cardell†, K. Riesbeck* & A. G. Wingren*

*Department of Medical Microbiology; and †Department of Otorhinolaryngology, Lund University, Malmö University Hospital, Malmö, Sweden

Received 9 July 2004; Accepted in revised form 1 November 2004

Correspondence to: Dr A. G. Wingren, Department of Medical Microbiology, Lund University, Malmö University Hospital, SE-205 02 Malmö, Sweden. E-mail: anette.gjorloff-wingren@mikrobiol.mas.lu.se

Abstract

The *Moraxella* immunoglobulin (Ig) D-binding protein (MID) induces a strong proliferative response in human peripheral blood IgD^+ B cells from adults isolated by positive selection using anti-CD19-conjugated microbeads. Here, we show that tonsillar B cells from children isolated with positive selection are unable to respond to MID stimulation. The proliferative response was very low or absent at various concentrations of MID tested and at different time points analysed, whereas the MID response of tonsillar B cells from adults isolated with positive selection was considerably higher. Tonsillar B cells from children isolated with positive selection responded to formalin-fixed preparations of *Moraxella catarrhalis* and *Staphylococcus aureus* Cowan strain I. In comparison to cells isolated with positive selection, a much higher proliferative response was recorded in tonsillar B cells from children isolated with negative selection, indicating that occupation of the CD19 molecule (i.e. positive selection) inhibited the response. Indeed, the addition of anti-CD19 monoclonal antibodies (MoAb) to MID-activated tonsillar B cells from children isolated with negative selection strongly inhibited the proliferative response. In contrast, anti-CD21 MoAb at the same concentration did only show a minor inhibition on the MID-induced response. Pre-incubation of tonsillar B cells isolated from children with anti-CD19 or anti-CD21 MoAb did not affect the binding of biotin-conjugated MID as analysed by flow cytometry. These results suggest that MID-activated tonsillar B cells from children have a strong requirement for signalling through the CD19 molecule. Future experiments will further reveal the importance of CD19 and possibly other molecules for optimal activation of tonsillar B cells isolated from both children and adults.

Introduction

The B-cell receptor (BCR) on naïve B cells is of low affinity because the antigen receptors are unmutated [1]. The BCR is linked to CD21 (complement receptor 2 or CR2) which in turn is in complex with CD19/TAPA-1/Leu-13. This complex is capable of lowering the threshold for activation through the BCR. CD21 and CD19 interact through extracellular and transmembrane regions [2]. CD19 is a B-cell-specific member of the immunoglobulin (Ig) superfamily expressed by pre-B cells from the time of heavy-chain rearrangement until plasma cell differentiation [3]. CD19 has no known ligand but mediates intracellular signalling after cross-linking with CD21 and the BCR [4]. Antigen-bound C3d, the final cleavage product of C3, cross-links CD21 to the BCR and enhances signalling through the antigen receptor by one to two orders of magnitude [5]. Moreover, cross-linking either CD19 or CD21 to limited numbers of membrane IgM on B cells enhances by 10–1000-fold the cellular responses, elevation of intracellular Ca^{2+}, activation of mitogen-activated protein (MAP) kinases and proliferation [6].

The signals through the B-cell antigen receptor are central to B-cell development and antigen response. Defective BCR signalling can result in impaired B-cell development, immunodeficiencies and predisposition of autoimmunity [7]. The mature, naïve B cell expresses both IgM and IgD molecules on its surface, which acts as the B-cell receptor for the antigen. IgD may play a critical role in humoral defence against pathogens undergoing rapid expansion and mutational drift upon entry in

the host [8]. This is supported by the delayed antibody production and affinity maturation in response to model T-cell-dependent antigens of IgD-deficient mice. IgD-secreting plasma cells are very sparse in the bone marrow, but numerous in nasal mucosa, adenoids, salivary and lachrymal glands and infected tonsils [9]. Interestingly, as much as 20% of tonsil plasma cells have been reported to produce IgD [10, 11]. The tonsils are lymphoid tissues located in the pharynx that play an important role in the host defence against invading antigens in the upper respiratory tract. Several studies have demonstrated that the adenoids and the tonsils are engaged in antibody responses to bacterial pathogens that have a tropism to the naso- or oropharynx, like *Streptococcus pneumoniae*, *Haemophilus influenzae* and β-haemolytic streptococci [12, 13]. The anatomical localization and histological organization of the tonsils are particularly suited for the capture of foreign material from epithelial surfaces. *Moraxella catarrhalis* is a pathogen involved in respiratory tract infections in both children and adults [14, 15]. *S. pneumoniae*, *H. influenzae* and *M. catarrhalis* are all able to cause acute otitis media in children. Interestingly, both *H. influenzae* and *M. catarrhalis* express outer membrane surface proteins that activate IgD^+ B lymphocytes by cross-linking IgD [16–18]. In fact, *M. catarrhalis* was already shown to display a strong affinity for soluble human IgD two decades ago [16].

A novel surface protein of *M. catarrhalis* designated MID for *Moraxella* IgD-binding protein was recently isolated in our laboratory [19]. The protein of approximately 200 kDa was found to bind to two purified IgD myeloma proteins, four IgD myeloma sera and one IgD standard serum. No binding of MID to IgG, IgM, IgA or IgE was detected [19]. Moreover, MID induced a strong proliferative response in human peripheral IgD^+ B lymphocytes [20]. The binding and subsequent activation of MID was IgD specific, and MID did neither bind to nor activate T cells or monocytes from human peripheral blood. IgM secretion was detected in B cells stimulated with MID and interleukin (IL)-2, whereas the secretion of IgG and IgA was induced by MID, IL-4, IL-10 and soluble CD40L. A truncated fragment of MID comprising 238 amino acid residues has been identified with preserved IgD binding ($MID^{962-1200}$) [21]. Interestingly, the IgD-binding capacity of a tetrameric structure of $MID^{962-1200}$ was 20-fold more efficient than the monomeric form, indicating that optimal binding of MID to IgD requires a tetramer.

In the present study, we have analysed the MID response of tonsillar B cells isolated from children and adults. Tonsillar B cells isolated from children with anti-CD19 monoclonal antibodies (MoAb) (positive selection) were unable to respond to IgD-mediated MID stimulation. In contrast, B cells from most adult donors isolated with positive selection responded to MID stimulation by proliferation. Most interestingly, tonsillar B cells from children isolated with negative selection could be induced to a strong proliferation in the presence of MID. Moreover, the proliferative response of MID-activated tonsillar B cells isolated from children with negative selection was inhibited in the presence of anti-CD19 MoAb, but not to the same extent by anti-CD21 MoAb. These results indicate that the CD19 molecule, either alone or in complex with CD21, is necessary for optimal MID activation through IgD and that tonsillar B cells from children have a stronger requirement compared to adults for costimulation through the CD19 molecule.

Materials and methods

Reagents. The purification of the *M. catarrhalis* outer membrane protein MID has been described previously [19]. Formalin-fixed preparations of *M. catarrhalis* strain Bc5 and *Staphylococcus aureus* strain Cowan I were titrated for optimal stimulation of tonsillar B lymphocytes. MoAb to the following antigens were used: mouse anti-human leucocyte antigen-DR (HLA-DR)–fluorescein isothiocyanate (FITC) and CD3–R-phycoerythrin (RPE) (Immunotech, Marseille, France), rabbit anti-human IgD–RPE, mouse-anti-human CD21, rabbit antimouse–FITC, rabbit anti-human CD19–FITC, rabbit anti-human CD19–RPE, streptavidin-RPE and rabbit-anti-human IgD–RPE from DAKO (Glostrup, Denmark). Mouse anti-human CD19 was from Serotec (Oxford, UK).

Biotinylation of MID. MID was conjugated with biotin using a FluoReporter® Mini-Biotin-XX protein-labelling kit (Molecular Probes, Oregon, OR, USA) according to the manufacturer's instructions. For the analysis of MID binding to membrane IgD on B cells, 20 μg/ml of MID–biotin was incubated with 4×10^5 tonsillar B cells for 20 min on ice. The cells were washed twice with 0.5% phosphate-buffered saline (PBS)–bovine serum albumin (BSA) and thereafter incubated for 20 min with streptavidin–RPE as a secondary step. After two washes in 0.5% PBS–BSA, the binding of MID–biotin to tonsillar B cells was analysed by flow cytometry (FACSCalibur, BD Biosciences, San Jose, CA, USA). When studying potential disturbance by anti-CD19 and anti-CD21 antibodies on MID binding to B cells, cells were first pre-incubated with the antibodies for 30 min on ice and thereafter washed twice in 0.5% PBS–BSA before incubating with MID–biotin as described above.

Cell preparations. Human tonsils were obtained from children (age 2–6 years) and adults (age >18 years) undergoing tonsillectomy at Malmö University Hospital (Ethical approvement No. LU 486-01). Briefly, tonsils were minced and cell suspension filtered through 70 μm nylon cell strainer (Becton Dickinson, Lincoln Park, NJ, USA) before Lymphoprep (Nycomed, Oslo, Norway) density-gradient centrifugation. B lymphocytes were isolated either by positive selection using anti-CD19-conjugated

magnetic beads or by negative selection using human B cell isolation kit II and thereafter anti-CD3-conjugated magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). A VarioMACS magnetic cell sorter (Miltenyi Biotec) was used for B-cell isolation according to the manufacturer's instructions. The positively selected B cells were routinely >97% HLA-DR$^+$. Negative selection of B cells allows a purification protocol without the addition of B-cell-specific MoAb. However, the final purity of negatively selected B cells is usually lower than 90% as measured by the number of HLA-DR$^+$ and CD3$^+$ T cells. To achieve higher than 97% purity of negatively selected B cells, we performed negative selection of B cells and thereafter anti-CD3-positive selection of the remaining T cells. The purity of the final negatively selected B-cell population was routinely >97% HLA-DR$^+$. B cells (2×10^{5}) from human tonsils were incubated in RPMI-1640 medium (Life Technologies, Paisley, UK) supplemented with 10% fetal calf serum, 50 μg/ml gentamicin and 200 U/ml penicillin (culture medium) and cultured in 96-well round-bottom plates (Nunc, Roskilde, Denmark) in triplicates in a final volume of 200 μl culture medium. Cell proliferation was measured routinely after 3 days (or as indicated) by [methyl-^{3}H]thymidine incorporation (5 μCi/well; Amersham Biosciences, Piscataway, NJ, USA) using an 18 h pulse period.

Detection of IL-6 by enzyme-linked immunosorbent assay (ELISA). IL-6 production was measured from cell supernatants harvested on day 3. In brief, ELISA plates (Maxisorp, Nunc) were coated with 50 μl of a solution containing rat anti-IL-6 antibody (2 μg/ml; Pharmingen, San Diego, CA, USA) diluted 1/1500 in 0.1 M Na_2HPO_4, pH 9.0. Standards and supernatants were diluted in PBS/Tween-20 (0.05%). Biotinylated rat anti-IL-6 antibody (1 μg/ml; BD PharMingen) was used as the secondary antibody, and thereafter HRP-conjugated avidin was added. Tetramethylbenzidine and hydrogen peroxide were used as chromogen and substrate. Finally, the absorbance at 405 nm was determined.

Statistic. A one-tailed t-test was used to determine the P-values.

Results and discussion

The IgD-mediated response of MID-stimulated tonsillar B cells from children isolated by positive selection using anti-CD 19-conjugated beads is very low or absent

We have recently shown that the novel outer membrane protein MID displays IgD-binding properties and is stimulatory for human IgD$^+$ peripheral blood B cells [20]. To optimize the response, soluble costimulatory molecules such as IL-4 and CD40 ligand (CD40L) was added to MID-stimulated peripheral blood B cells. In the present study, tonsillar B cells from children and adults were isolated either by positive selection using anti-CD19-conjugated magnetic beads or by negative selection. The purified B cells were thereafter subjected to stimulation with various concentrations of MID. The children analysed in this study were aged between 2 and 6 years and the adults were 18 years or older.

Tonsillar B cells from both children and adults were subjected to purification by positive selection using anti-CD19 beads. The concentration of MID that induced a maximal response in IgD$^+$ peripheral blood B cells [20] and in tonsillar B cells isolated from adults was 0.5 μg/ml, whereas a much lower response was seen in tonsillar B cells isolated from children when stimulated with the same concentration of MID (Fig. 1A–C). As shown in Fig. 1A, the proliferative response of tonsillar B cells isolated from children was very low or absent at all concentrations of MID tested, whereas the response of tonsillar B cells isolated from adults was high and comparable to that seen in IgD$^+$ peripheral blood B cells [20]. In total, tonsillar B cells from 14 children and 18 adults were isolated and purified by positive selection and thereafter activated with 0.5 μg/ml of MID. The proliferative response of adult tonsillar B cells was significantly higher compared to the response of B cells isolated from children (Fig. 1B). A sixfold increase was detected in the proliferative response and the IL-6 production of MID-stimulated tonsillar B cells isolated from adults compared to children (Fig. 1B,C). Interestingly, B cells isolated from children with positive selection using anti-CD19 beads responded to a formalin-fixed preparation of *M. catarrhalis* (MCat) (Fig. 1D) and to a formalin-fixed preparation of *S. aureus* Cowan strain I (S. aur.) (Fig. 1E). However, the response in B cells isolated from adults was significantly higher.

The absence of the IgD-mediated MID response in tonsillar B cells from children purified by anti-CD19-conjugated beads is not due to a different kinetics

B cells purified by positive selection from children and adults were stimulated with 0.5 μg/ml of MID and incubated during different time points as indicated (Fig. 2). The result shows that MID induced a very weak response in tonsillar B cells isolated from children and purified by positive selection at all time points analysed, whereas the response of tonsillar B cells isolated from adults was high.

Isolation of tonsillar B cells from children by negative selection results in a strong IgD-mediated proliferative response

Tonsillar lymphocytes isolated from three children were split in half before purification and purified either by positive selection using anti-CD19-conjugated beads or negative selection. The proliferative response of MID-stimulated

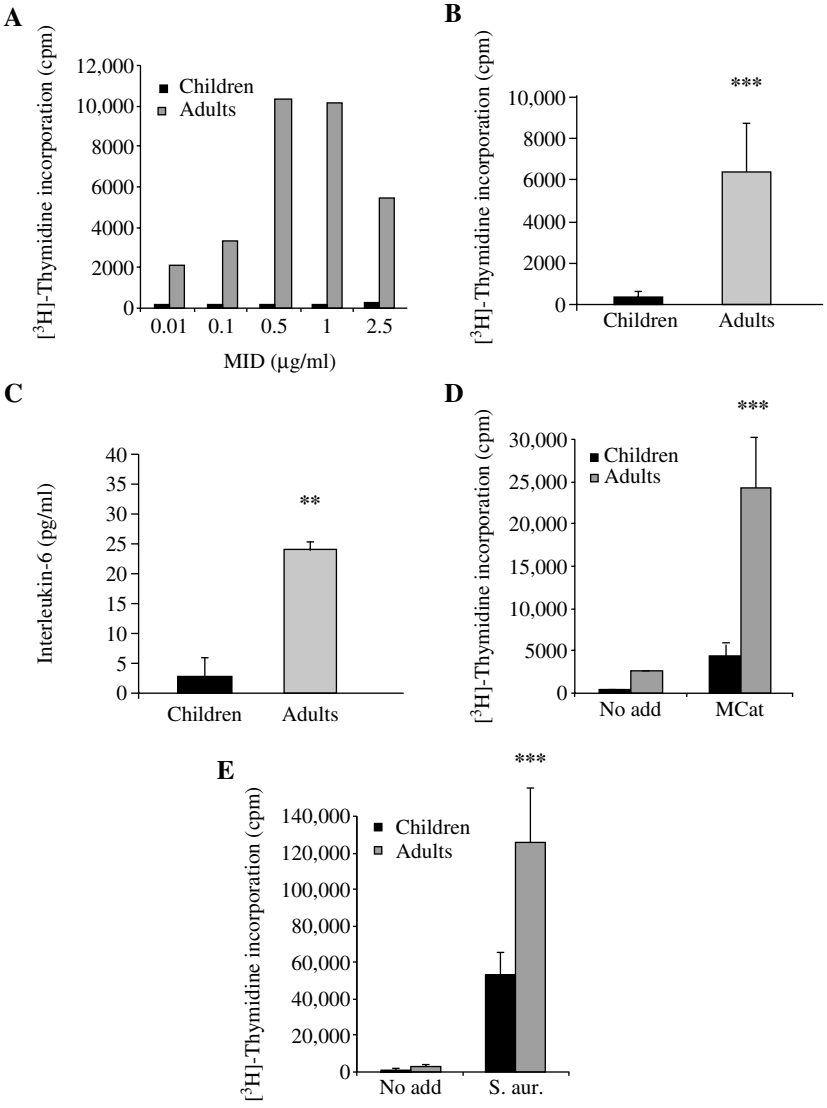

Figure 1 Human tonsillar B cells from children isolated with positive selection using anti-CD19 microbeads are unresponsive to *Moraxella* immunoglobulin (Ig) D-binding protein (MID). Purified $CD19^+$ tonsillar B cells (1×10^6 cells/ml) from children and adults were incubated in culture medium alone (no add) or with MID concentrations as indicated, formalin-fixed preparation of *M. catarrhalis* (MCat) (1 : 2000 dilution) or *S. aureus* Cowan strain I (S. aur.) (1 : 8000 dilution). [*Methyl*-^{3}H]thymidine (5 μCi/well) uptake was measured after 3 days of activation. (A) Results are presented as one representative experiment out of four (children) and two (adults) performed. (B) Results are presented as the mean value ± SEM from 14 children and 18 adults tested. (C) $CD19^+$ tonsillar B cells (1×10^6 cells/ml) from children and adults were stimulated in culture medium with 0.5 μg/ml MID. Supernatants were harvested after 3 days of activation and analysed for IL-6 secretion by enzyme-linked immunosorbent assay. Results are presented as the mean values ± SEM from four children and two adults analysed. (D and E) Results are presented as the mean value ± SEM from 12 children and 10 adults tested. $^{***}P < 0.005$, $^{**}P < 0.01$.

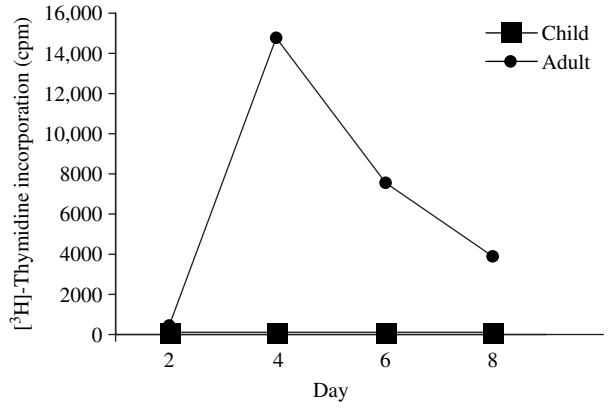

Figure 2 Unresponsiveness to *Moraxella* immunoglobulin (Ig) D-binding protein (MID) is not due to a difference in kinetics. $CD19^+$ tonsillar B cells (1×10^6 cells/ml) from children and adults were stimulated in culture medium with 0.5 μg/ml of MID. [*Methyl*-^{3}H]thymidine (5 μCi/well) uptake was measured after 2, 4, 6 and 8 days of activation. Results are presented as one representative experiment out of two performed.

tonsillar B cells isolated with positive selection was low (Fig. 3A), whereas the response in tonsillar B cells isolated with negative selection was considerably higher (Fig. 3B). These results indicate that occupation of the CD19 molecule (i.e. positive selection) inhibits IgD-mediated stimulation. The proliferative response induced by MCat, a formalin-fixed preparation of *M. catarrhalis*, was also higher in tonsillar B cells from children after isolation with negative selection (Fig. 3B). The response induced by *S. aureus*, a formalin-fixed preparation of *Staphylococcus aureus* Cowan strain I, was high in B cells isolated with positive selection, but increased further in B cells isolated by negative selection. Indeed, several studies have shown that IgM stimulation is enhanced by costimulation through CD19 [3, 5]. To our knowledge, this is the first time it is shown that CD19 is an important coreceptor for IgD-mediated stimulation.

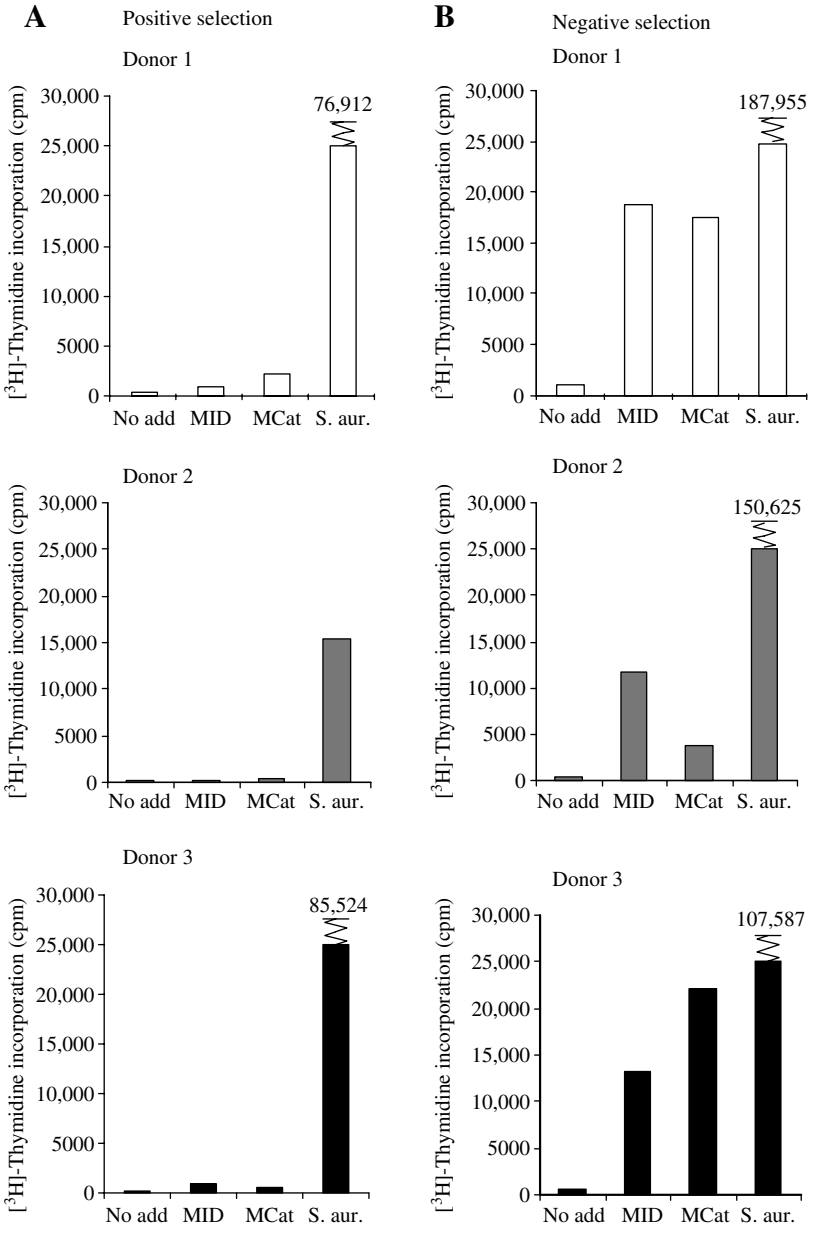

Figure 3 *Moraxella* immunoglobulin (Ig) D-binding protein (MID) activation of tonsillar B cells from children isolated with negative selection results in a strong proliferative response. Tonsillar B cells (1×10^6 cells/ml) from three children were isolated using positive selection with anti-CD19 microbeads (A) or negative selection (B) and incubated in culture medium alone (no add) or with MID (0.5 μg/ml), formalin-fixed preparation of *M. catarrhalis* (MCat) (1 : 2000 dilution) or *S. aureus* Cowan strain I (S. aur.) (1 : 8000 dilution). [*Methyl*-^{3}H]thymidine (5 μCi/well) uptake was measured after 3 days of activation. Results are presented as three representative experiments out of four performed.

Blocking experiments with anti-CD19 antibody reveal the importance of this costimulatory molecule for optimal MID activation of tonsillar B cells from children

CD19 is physically associated with CD21 in the cell membrane. After positive selection of B cells using anti-CD19-conjugated beads, not only the CD19 molecule but also the CD21 molecule is occupied for anti-CD21 MoAb staining (our own observation). We therefore aimed to block the MID-induced proliferation of negatively selected B cells by adding either anti-CD19 or anti-CD21 MoAb to the culture. Indeed, the addition of anti-CD19 MoAb strongly inhibited the proliferation of MID-activated B cells from children (Fig. 4). The addition of anti-CD21 MoAb only had a minor inhibitory effect on the MID-induced proliferation of B cells in children (Fig. 4). Similar results were obtained in adults when blocking with anti-CD19 antibody, indicating the importance of this molecule in older age groups as well (data not shown).

Here, we demonstrate that protein MID and MCat are unable to induce an optimal response in IgD^+ tonsillar B cells from children isolated by positive selection using anti-CD19-conjugated beads. In contrast, tonsillar B cells from the same donors isolated with negative selection show a 10- to 20-fold increase in the response to MID and MCat. Our results suggest that the CD19 molecule is crucial for the IgD-binding protein MID to stimulate B cells.

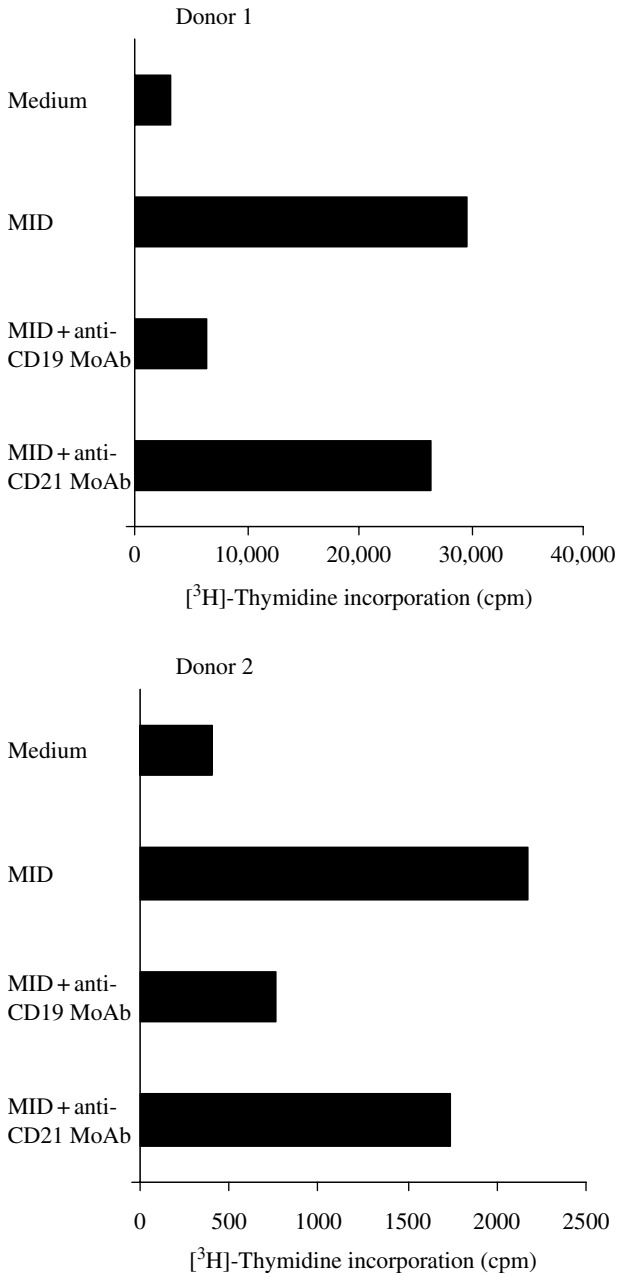

Figure 4 The CD19 molecule is important for an optimal *Moraxella* immunoglobulin (Ig) D-binding protein (MID) response in tonsillar B cells from children. Tonsillar B cells (1×10^6 cells/ml) from children purified by negative selection were incubated in culture medium alone (no add), or with MID (0.5μg/ml) in combination with or without 10 μg/ml of mouse anti-human CD19 antibody or 10 μg/ml of mouse anti-human CD21 antibody as indicated. [*Methyl*-^{3}H]thymidine (5 μCi/well) uptake was measured after 3 days of activation. Results are presented as two representative experiments out of two performed. MoAb, monoclonal antibody.

MID-induced proliferation and IL-6 production are totally inhibited in tonsillar B cells isolated from most children when the CD19 molecule is physically blocked. This is true when using positive selection with anti-CD19-conjugated beads as the purification method as well as when adding anti-CD19 MoAb in culture to MID-activated B cells isolated with negative selection. Interestingly, blocking with anti-CD21 MoAb did not have such dramatic effect (Fig. 4). As CD21 has a very short cytoplasmic domain without signalling function, CD21 most likely serves as a ligand-binding subunit that links the recognition of antigen to the signalling function of CD19 [6].

Anti-CD19 or anti-CD21 MoAb do not block IgD targeting of protein MID

We next evaluated whether the binding of MID to IgD was affected by pre-incubation of anti-CD19 or anti-CD21 MoAb. Tonsillar B cells isolated from children with negative selection were pre-incubated with MoAb against CD19 or CD21. Thereafter, biotin-conjugated MID was added and binding analysed by flow cytometry. As shown in Fig. 5, pre-incubation with anti-CD19 MoAb (Serotec) or anti-CD21 MoAb did not inhibit the binding of MID to tonsillar B cells isolated from children. Moreover, another anti-CD19 antibody (rabbit-anti-human CD19-RPE from DAKO) was tested for the ability to block the binding of MID, which also showed a similar result (data not shown). We are currently investigating the binding of MID to different human IgD^+CD19^+ B-cell lines. Preliminary results obtained by flow cytometry analysis reveal that the presence of CD21 is crucial for MID binding to IgD^+CD19^+ B cells. B-cell lines that express IgD and CD19, but lack or express very low levels of CD21, bound the MID protein to a lesser extent compared to cells that express IgD, CD19 and moderate or high levels of CD21. As the anti-CD21 MoAb used in our study was unable to inhibit MID-induced proliferation as well as binding, it might be speculated that the CD21-binding epitope of this MoAb does not interfere with the MID-binding epitope of CD21. Whether or not MID-mediated signalling through CD19 is dependent on CD21 needs to be further evaluated.

For the membrane-bound BCR, Igα (CD79a) and Igβ (CD79b) play essential roles in the signal transduction through the immunoreceptor tyrosine-based activation motifs (ITAMs) located in their intracytoplasmic region. It is thought that signals transduced by IgM or IgD receptors on B cells are the same but with different kinetics [22]. The affinity threshold required for IgM signalling is decreased when antigen–C3d complexes colligate the BCR and CD19/CD21 complex [1]. It is obvious that CD19 signalling complements or modulates the regulatory signals of other receptors, particularly the BCR. However, the signalling capability of CD19 is not dependent on the expression of surface immunoglobulin, as CD19 ligation can provide proliferative signals for surface IgM^- precursor B cells [23]. CD21 is physically associated with CD19 on the surface of human B cells, but the CD19 complex does

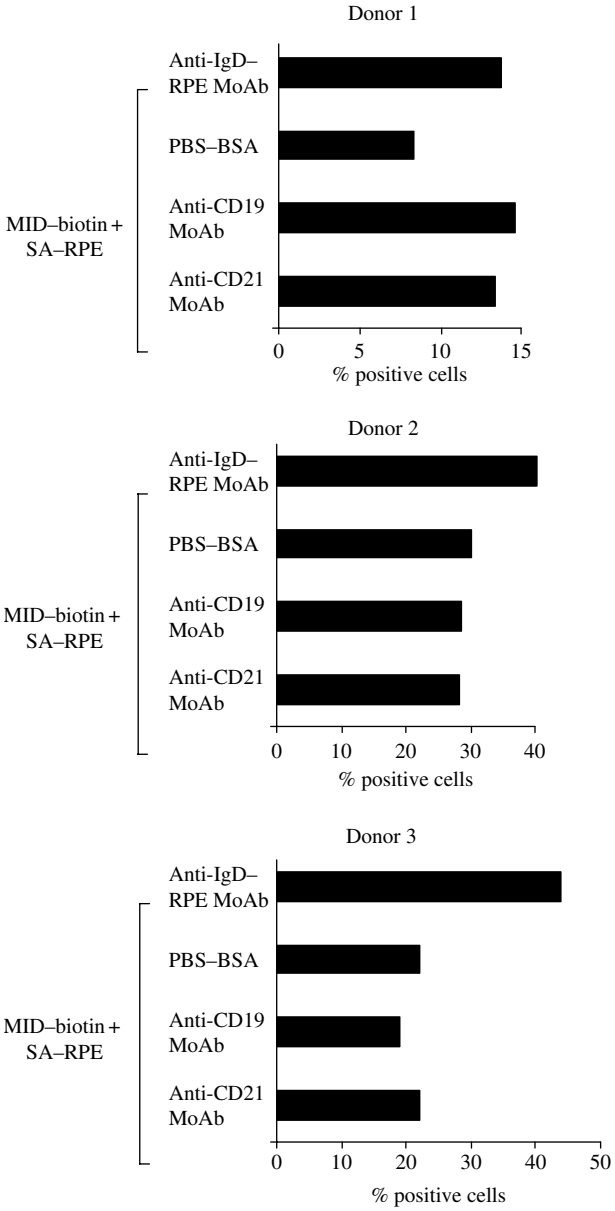

Figure 5 The binding of *Moraxella* immunoglobulin (Ig) D-binding protein (MID) to tonsillar B cells is not disturbed by anti-CD19 or anti-CD21 monoclonal antibody (MoAb). Tonsillar B cells from children isolated with negative selection were analysed by flow cytometric analysis. Cells (4×10^5 cells) were incubated with 20 μg/ml of biotin-conjugated MID and thereafter with streptavidin (SA)–R-phycoerythrin(RPE) as a secondary step, with or without pre-incubation with 10 μg/ml mouse anti-human CD19 MoAb or mouse anti-human CD21 MoAb as indicated. B cells from each donor were also labelled with rabbit anti-human IgD–RPE. Results are presented as percentage of positive cells specific for the antibody analysed. The background level (no antibody added) was set to <1% positive cells. Results are presented as three representative experiments out of four performed. PBS, phosphate-buffered saline; BSA, bovine serum albumin.

not require CD21 for signal transduction. After the isolation of B cells using anti-CD19-conjugated beads, not only the CD19 molecule, but also the CD21 molecule is occupied for anti-CD21 antibody staining (our own observation). CD21 binds to the complement fragments iC3b, C3d and/or C3dg, which can function as opsonins and stimulate phagocytosis or, in the case of B cells, act as a link between costimulatory receptors and the BCR.

Moraxella catarrhalis has now been recognized as an important pathogen in respiratory tract infections in both children and adults [14, 15]. *M. catarrhalis* is the third most common bacterial agent in acute otitis media in children and often implicated as a cause of sinusitis in both children and adults. The search for potential vaccine antigens is an important task, as more than 90% of *M. catarrhalis* clinical isolates are resistant to penicillin. It is therefore of great importance to study the interactions between IgD and IgD-binding proteins, especially in children. Young children have a higher vulnerability to infections mainly due to immaturity of the immune system, but one of the main problems in infant immunity is the inability to mount an adequate and rapid response to thymus-independent type 2 (TI-2) antigens (partly dependent on T-cell help) [24, 25]. It has been shown that children under 2 years of age lack the expression of CD21 on marginal zone B cells in the spleen [25]. In our study, tonsillar B cells from children aged between 2 and 6 years expressed similar amounts of CD21 as compared with tonsillar B cells from adults (data not shown). Moreover, the addition of anti-CD21 MoAb to negatively selected MID-stimulated B cells from children only have a minor effect on the proliferation, whereas anti-CD19 MoAb strongly inhibited the proliferative response. The complement receptor 1 (or CD35) is also expressed on human B cells and binds C3b fragments. CD21 and CD35 might be physically associated on the surface. Most interestingly, Jozsi *et al.* [26] have shown that CD35 mediates inhibitory signals in tonsillar B cells from children. The role of the molecule in B-cell activation is controversial, probably due to different experimental conditions, the use of mixed cell populations and antibodies that react with various epitopes of CD35. Whether CD35 also has a role in IgD-mediated signalling in tonsillar B cells of children will be further investigated in our laboratory.

In conclusion, the bacterial outer membrane protein MID binds with strong affinity to human IgD, but requires the CD19 molecule either alone or in complex with CD21 for the induction of optimal activation of tonsillar B cells. Importantly, tonsillar B cells from children seem to have a strong requirement for the costimulatory CD19-signalling pathway when stimulated with MID.

Acknowledgment

This work was supported by grants from the Alfred Österlund Foundation, the Crafoord Foundation, the Greta and

Johan Kock Foundation, the Magnus Bergvall Foundation, the Medical Faculty of Lund, the Swedish Society of Medicine, the Swedish Medical Research Council and the Cancer Foundation at Malmö University Hospital.

References

1 Fearon DT, Carter RH. The CD19/CR2/TAPA-1 complex of B lymphocytes: linking natural to acquired immunity. Annu Rev Immunol 1995;13:127.

2 Zandvoort A, Timens W. The dual function of the splenic marginal zone: essential for initiation of anti-TI-2 responses but also vital in the general first-line defense against blood-borne antigens. Clin Exp Immunol 2002;130:4.

3 Sato S, Steeber DA, Jansen PJ, Tedder TF. CD19 expression levels regulate B lymphocyte development: human CD19 restores normal function in mice lacking endogenous CD19. J Immunol 1997;158:4662.

4 Carroll MC. The role of complement and complement receptors in induction and regulation of immunity. Annu Rev Immunol 1998;16:545.

5 Carter RH, Spycher MO, Ng YC, Hoffman R, Fearon DT. Synergistic interaction between complement receptor type 2 and membrane IgM on B lymphocytes. J Exp Med 1988;167:1047.

6 Fearon DT, Carroll MC. Regulation of B lymphocyte responses to foreign and self-antigens by the CD19/CD21 complex. Ann Rev Immunol 2000;18:393.

7 Gauld SB, Dal Porto JM, Cambier JC. B cell antigen receptor signaling: roles in cell development and disease. Science 2002;296:1641.

8 Roes J, Rajewsky KJ. Immunoglobulin D (IgD)-deficient mice reveal an auxiliary receptor function for IgD in antigen-mediated recruitment of B cells. J Exp Med 1993;177:45.

9 Preud'homme JL, Petit I, Barra A, Morel F, Lecron JL, Lelievre E. Structural and functional properties of membrane and secreted IgD. Mol Immunol 2000;37:871.

10 Liu YJ, de Bouteiller O, Arpin C *et al.* Normal human IgD^+ IgM^- germinal center B cells can express up to 80 mutations in the variable region of their IgD transcripts. Immunity 1996;4:603.

11 Arpin C, de Bouteiller O, Razanajaona D *et al.* The normal counterpart of IgD myeloma cells in germinal center displays extensively mutated IgVH gene, Cmu-Cdelta switch and lambda light chain expression. J Exp Med 1998;187:1169.

12 Harabuchi Y, Hamamoto M, Shirasaki H, Asakura K, Matsuyama H, Kataura A. Specific immune response of the adenoids to a respiratory antigen. Am J Otolaryngol 1989;10:138.

13 Rynell-Dagoo B. The immunological function of the adenoid. Acta Otolaryngol 1976;82:196.

14 Catlin BW. *Branhamella catarrhalis*: an organism gaining respect as a pathogen. Clin Microbiol Rev 1990;3:293.

15 Karalus R, Campagnari A. *Moraxella catarrhalis*: a review of an important human mucosal pathogen. Microbes Infect 2000;2:547.

16 Forsgren A, Grubb AO. Many bacterial species bind human IgD. J Immunol 1979;122:1468.

17 Forsgren A, Penta A, Schlossman S, Tedder TF. *Branhamella catarrhalis* activates human B lymphocytes following interactions with surface IgD and class I major histocompatibility complex antigens. Cell Immunol 1988;112:78.

18 Brandtzaeg P. Humoral immune response patterns of human mucosae: induction and relation to bacterial respiratory tract infections. J Infect Dis 1992;165:167.

19 Forsgren A, Brant M, Möllenkvist A *et al.* Isolation and characterization of a novel IgD-binding protein from *Moraxella catarrhalis*. J Immunol 2001;167:2112.

20 Gjörloff Wingren A, Hadzic R, Forsgren A, Riesbeck K. The novel IgD binding protein from *Moraxella catarrhalis* induces human B lymphocyte activation and Ig secretion in the presence of Th2 cytokines. J Immunol 2002;168:5582.

21 Nordström T, Forsgren A, Riesbeck K. The immunoglobulin D-binding part of the outer membrane protein MID from *Moraxella catarrhalis* comprises 238 amino acids and a tetrameric structure. J Biol Chem 2002;277:34692.

22 Vladutiu AO. : Immunoglobulin D: properties, measurement and clinical relevance. Clin Diagn Lab Immunol 2000;7:131.

23 Tedder TF, Inaoki M, Sato S. The CD19–CD21 complex regulates signal transduction thresholds governing humoral immunity and autoimmunity. Immunity 1997;6:107.

24 Timens W, Boes A, Rozeboom T, Poppema S. Immaturity of the human splenic marginal zone in infants: possible contribution to the deficient infant immune response. J Immunol 1989;143:3200.

25 Zandvoort A, Lodewijk ME, de Boer NK, Dammers PM, Kroese FGM, Timens W. CD27 expression in the human splenic marginal zone: the infant marginal zone is populated by naive B cells. Tissue Antigens 2001;58:234.

26 Jozsi M, Prechl J, Bajtay Z, Erdei A. Complement receptor type 1 (CD35) mediates inhibitory signals in human B lymphocytes. J Immunol 2002;168:2782.