Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 345 (2006) 474–478
BBRC
www.elsevier.com/locate/ybbrc

Molecular characteristics of IgA and IgM Fc binding to the Fcα/μR

Yukiko Cho [1], Kenta Usui [1], Shin-ichiro Honda [1], Satoko Tahara-Hanaoka, Kazuko Shibuya, Akira Shibuya *

Department of Immunology, Institute of Basic Medical Sciences, Graduate School of Comprehensive Human Sciences and Center for TARA, University of Tsukuba, 1-1-1 Tennodai, Tsukuba Science City, Ibaraki 305-8575, Japan

Received 11 April 2006
Available online 27 April 2006

Abstract

Fcα/μ receptor (Fcα/μR), a novel Fc receptor for IgA and IgM, is a type I transmembrane protein with an immunoglobulin (Ig)-like domain in the extracellular portion. Although IgA and IgM bind to Fcα/μR, the molecular and structural characteristics of the ligand-receptor interactions have been undetermined. Here, we developed twelve monoclonal antibodies (mAbs) against murine Fcα/μR by immunizing mice deficient in *Fcα/μR* gene. Eight mAbs totally or partially blocked IgA and IgM bindings to Fcα/μR. These blocking mAbs bound to a peptide derived from the Ig-like domain of murine Fcα/μR, which is conserved not only in human and rat Fcα/μR but also in polymeric Ig receptor (poly-IgR), another Fc receptor for IgA and IgM. These results suggest that IgA and IgM bind to an epitope in the conserved amino acids in the Ig-like domain of Fcα/μR as well as poly-IgR.

Keywords: Fcα/μR; Fc receptor; IgM; IgA; Polymeric immunoglobulin receptor; Immunoglobulin-like domain

The receptors for Fc portions of immunoglobulins (FcR) mediate various immune responses upon binding to antibodies or immune complexes [1,2]. Mice deficient in FcR genes for IgG (*FcgRI*, *FcgRII*, and *FcgRIII*) and IgE (*FceRI* and *FceRII*) have revealed their physiological roles in immune responses in vitro and in vivo [3,4]. Although functional Fc receptors for IgM had been reported on subpopulations of human and rodent T, B, and NK cells [5–10], a gene encoding an Fcμ receptor had not previously been identified. We finally cloned a novel Fc receptor for IgM, which was found to be also an Fc receptor for IgA, designated Fcα/μ receptor (Fcα/μR) [11,12]. The Fcα/μR is a type I transmembrane protein with an immunoglobulin (Ig)-like domain in the extracellular portion, and is expressed on B cells and macrophages [11,12]. The Fcα/μR is an only IgM receptor identified on human and murine hematopoietic cells to date, and is thought to play a central role for immune responses mediated by IgM and IgA [13].

The Fcα/μR genes were mapped to syntenic regions of mouse chromosome 1 (1F) and human chromosome 1 (1q32.3), near several other Fc receptors, including Fcγ receptors I, II, and III, Fcε receptor, and the polymeric Ig receptor (poly-IgR) [11]. The poly-IgR gene, another Fc receptor for IgM and IgA expressed on mucosal epithelial cells [14], is only 12 kbp apart from the Fcα/μR in the Fc receptor gene cluster on chromosome 1 [15], suggesting that these two receptors seem to be closely related in their phylogenies. The poly-IgR contains five Ig-like domains in the extracellular portion. We found a motif in the immunoglobulin (Ig)-like domain of human and murine Fcα/μR that is conserved in the first Ig-like domain of human, mouse, and rat poly-IgR [11]. Although this region is important for IgM and IgA binding to the poly-IgR [14], the molecular and structural characteristics of IgA and IgM binding to Fcα/μR have been unclear.

We have recently established twelve monoclonal antibodies (mAbs) against murine Fcα/μR (mFcα/μR), some of which efficiently block the IgM and IgA bindings to

* Corresponding author. Fax: +81 29 853 3410.
E-mail address: ashibuya@md.tsukuba.ac.jp (A. Shibuya).
[1] These three authors equally contributed to this work.

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.04.084

Fcα/μR. By using these mAbs, we analyzed the molecular characteristics of the ligand binding by Fcα/μR.

Materials and methods

Cells, mice, antibodies, and peptides. Ba/F3 and BW5147 are mouse pro-B and thymoma cell lines, respectively. The Ba/F3 and BW5147 transfectants stably expressing mFcα/μR were established, as described [11]. Mice deficient in *Fcα/μR* gene were established by using gene targeting strategy of ES cells (manuscript under preparation). Fluorescein isothiocyanate (FITC)-conjugated streptavidin, mouse IgM and IgA were purchased from PharMingen (San Diego, CA). The peptides generated based on a motif in the Ig-like domain of mFcα/μR (VTIHCHYAPSSVNRHQRKYW) [11] and chicken egg ovalbumin (OVA) (ISQAVHAAHAEINEAGR) were purchased from Peptide Institute, Inc. (Osaka, Japan).

Establishment of monoclonal antibodies against mFcα/μR. Ba/F3 (1×10^7) transfectant expressing mFcα/μR emulsified with complete Freund adjuvant (Sigma, St. Louis, MO) was injected into both foot pads of an Fcα/μR-deficient mouse on days 0 and 7. On day 10, draining lymph node cells were harvested and fused with Sp2/0 myeloma cells. Hybridomas were grown on methyl cellurose plate (ClonaCell-HY^R, Stem Cell Technologies, Seattle, WA), according to the manufacturer's instruction.

Competitive binding analyses. To compare recognition sites by each anti-mFcα/μR mAb generated one another, 1×10^6 BW5147 transfectants expressing mFcα/μR were incubated with a variable dose of each anti-mFcα/μR mAb generated for 30 min at 4 °C. The transfectants were then washed with PBS and incubated with 0.05 μg of a biotin-conjugated anti-mFcα/μR mAb, followed by FITC-conjugated streptavidin.

To examine blocking ability for IgM and IgA bindings to the mFcα/μR, 1×10^6 BW5147 transfectants expressing mFcα/μR were incubated with 0.5 μg of each purified anti-mFcα/μR mAb for 30 min at 4 °C, washed with PBS twice, and then stained with FITC-conjugated mouse IgM or IgA.

To examine binding ability of anti-Fcα/μR mAbs to the motif peptide conserved in Fcα/μR and poly-IgR, 0.05 μg of each biotin-conjugated anti-mFcα/μR mAb was incubated with a variable dose of either motif peptide or control peptide for 1 h at 4 °C. Then 1×10^6 BW5147 transfectants expressing mFcα/μR were added into the mAbs-peptide mixture and incubated for 30 min at 4 °C, followed by FITC-conjugated streptavidin. The transfectants were analyzed by FACS Calibur^R (BD, San Diego, CA).

Results and discussion

Establishment of mAbs against the mFcα/μR

Although we had been trying to generate mAbs against mFcα/μR by immunizing rats with the Ba/F3 transfectant expressing mFcα/μR several times, we could generate only one anti-mFcα/μR mAb (TX6) [11]. Furthermore, we could never generate anti-human Fcα/μR (hFcα/μR) mAb by immunizing mice with the hFcα/μR antigens. Because amino acid sequences of extracellular domain of Fcα/μR are highly conserved among human, mouse, and rat (80.2% identity between mouse and rat and 51.3% between mouse and human) [11], we considered that the immunological tolerance against Fcα/μR antigens may be a cause of difficulty for establishing anti-human and murine Fcα/μR mAbs. In order to avoid immunological tolerance against mFcα/μR antigens, we immunized a mouse

Table 1
Summary of the characteristics of anti-mFcα/μR mAbs

Group	I	II	III					IV		V		
	TX57	TX64	TX58	TX59	TX60	TX61	TX66	TX67	TX68	TX62	TX63	TX65
Isotype	IgG1	IgG1	IgG1	IgG1	IgG1	IgG1	IgG1	IgG1	IgG1	IgG2a	IgG2a	IgG2a
Blocking capacity of IgM binding	++	–	+	+	+	+	+	+	+	–	–	–
Blocking capacity of IgA binding	++	–	+	+	+	+	+	+	+	–	–	–
Cross-reactivity to human Fcα/μR	–	–	+	+	+	+	+	–	–	–	–	–
Binding to the motif peptide	+	–	+	+	+	+	+	+	+	–	–	–

See detail in the text, Table 2 and Figs. 1–3.

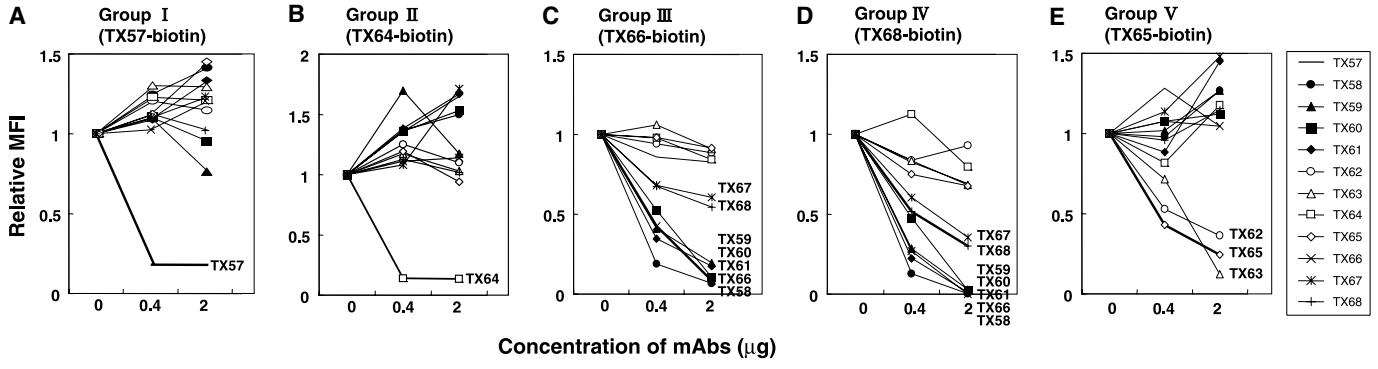

Fig. 1. Competitive binding analyses between anti-mFcα/μR mAbs. BW5147 transfectant expressing mFcα/μR was preincubated with each mAb and then stained with biotinylated mAb indicated, followed with FITC-conjugated streptavidine and analyzed by flow cytometry. Data are shown in relative mean fluorescence intensity (MFI) of transfectant stained with biotinylated antibodies after preincubation with each mAb, as compared with that stained with biotynyated antibodies alone. Data are representative from more than three independent experiments.

deficient in *Fcα/μR* gene, instead of rat or other species of animals, with the mFcα/μR-expressing Ba/F3 transfectant and successfully generated twelve anti-mFcα/μR mAbs, designated TX57 to TX68 (Table 1).

Characterization of recognition sites by the anti-mFcα/μR mAbs

To characterize recognition sites by the anti-mFcα/μR mAbs, we performed competitive binding analysis, by using the BW5147 transfectant expressing mFcα/μR. The BW5147 transfectant was incubated with either mAb out of 12 mAbs generated and then stained with either biotinylated mAb, followed by FITC-conjugated streptavidin. Analyses by flow cytometry demonstrated that any of mAbs, except TX57, did not interfere the binding of TX57 to the transfectant (Fig. 1A). Similarly, TX64 binding to the transfectant was not inhibited by any of mAbs other than TX64 itself (Fig. 1B). These results suggest that TX57 and TX64 mAbs recognize epitopes different from those recognized by other mAbs. In contrast, TX58, TX59, TX60, TX61, TX66, TX67, and TX68 mAbs, but not other mAbs, completely or partially inhibited the binding of these mAbs one another to the

Table 2
Competitive binding analyses between anti-mFcα/μRmAbs

Group	I	II	III					IV		V		
	TX57	TX64	TX58	TX59	TX60	TX61	TX66	TX67	TX68	TX62	TX63	TX65
Bio-TX57	++	–	–	–	–	–	–	–	–	–	–	–
Bio-TX64	–	++	–	–	–	–	–	–	–	–	–	–
Bio-TX58	–	–	++	++	++	++	++	+	+	–	–	–
Bio-TX59	–	–	++	++	++	++	++	+	+	–	–	–
Bio-TX60	–	–	++	++	++	++	++	+	+	–	–	–
Bio-TX61	–	–	++	++	++	++	++	+	+	–	–	–
Bio-TX66	–	–	++	++	++	++	++	+	+	–	–	–
Bio-TX67	–	–	++	++	++	++	++	++	++	–	–	–
Bio-TX68	–	–	++	++	++	++	++	++	++	–	–	–
Bio-TX62	–	–	–	–	–	–	–	–	–	++	++	++
Bio-TX63	+	–	–	–	–	–	+	–	–	++	++	++
Bio-TX65	–	–	–	–	–	–	–	–	–	++	++	++

BW5147 transfectant expressing mFcα/μR was preincubated with each mAb and then stained with biotinylated mAb indicated, followed with FITC-conjugated streptavidine and analyzed by flow cytometry. Reductions of mean fluorescence intensity (MFI) of transfectant stained with biotynyated antibodies after preincubation with each mAb, as compared with those without preincubation with any mAbs, were determined. (++, >50% reduction; +, 30–50% reduction; –, <30% reduction).

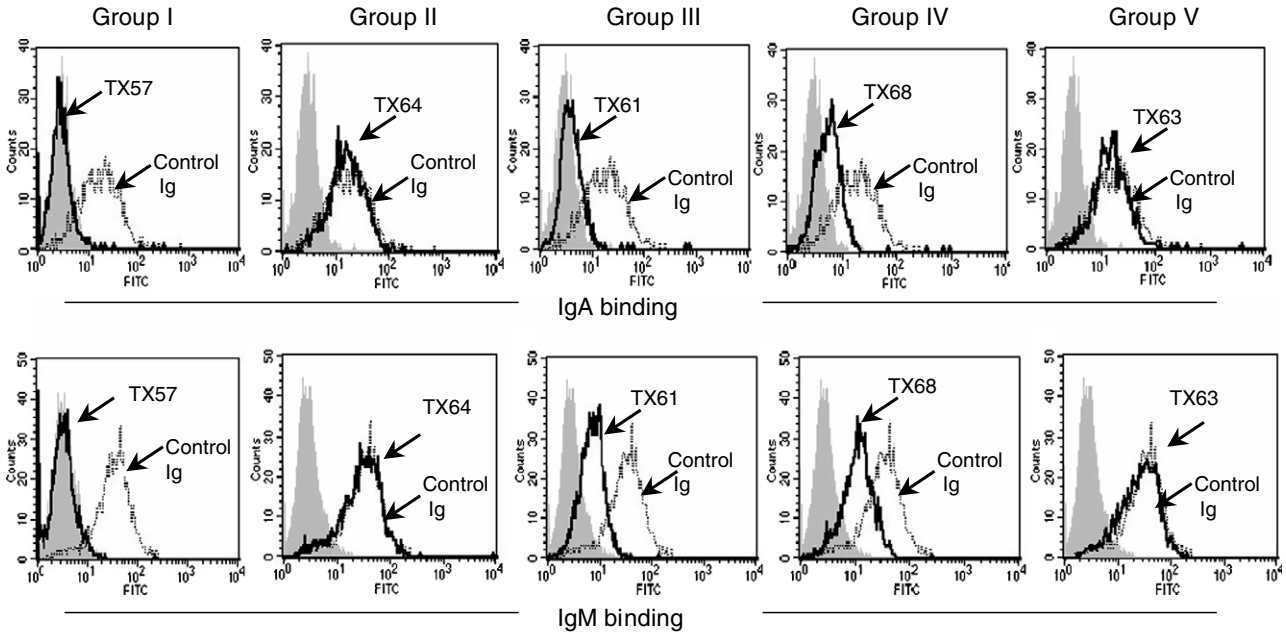

Fig. 2. Competitive binding analyses between anti-mFcα/μR mAbs and IgA or IgM. BW5147 transfectant expressing mFca/mR was pre-incubated with each mAb indicated (bold lines) or control Ig (dotted lines) and then stained with FITC-conjugated mouse IgA or IgM and analyzed by flow cytometry. Shaded histograms indicate autofluorescence of the transfectant. Data are representative from more than three independent experiments.

transfectant, respectively (Fig. 1C, D and Table 2). Similarly, TX62, TX63, and TX65 mAbs, but not other mAbs, interfered in the binding of these mAbs one another to the transfectant (Fig. 1E and Table 2). It was of interest that TX58, TX59, TX60, TX61, and TX66 mAbs, but not the others, cross-reacted with hFcα/μR (Table 1 and data not shown), suggesting that these mAbs recognize an epitope conserved also in human Fcα/μR. Based on these results, we divided the mAbs into five groups that differed from one other in their recognition sites (Table 1).

Blocking of ligand binding to the Fcα/μR by the anti-Fcα/μR mAbs

Next, we examined whether IgM and IgA bindings to the mFcα/μR could be blocked by the anti-mFcα/μR mAbs. The BW5147 transfectant expressing the mFcα/μR was incubated with each anti-mFcα/μR mAb and then further incubated with FITC-conjugated IgM or IgA. As demonstrated in Fig. 2 and Table 1, analyses by flow cytometry demonstrated that TX57 mAb in the group I totally inhibited the binding of both IgM and IgA to the transfectant. All the mAbs in the groups III and IV (TX58, 59, 60, 61, 66, 67, and 68) partially inhibited the ligand binding. In contrast, the mAbs in the groups II and V did not affect IgM and IgA bindings to the transfectant. These results suggested that the epitopes in the Fcα/μR recognized by the mAbs in the groups I, III, and IV are either the IgA and IgM binding site of the Fcα/μR or physically related to the ligand binding site.

Binding to a peptide conserved in both Fcα/μR and poly-IgR by the anti-mFcα/μR mAbs

We previously found a motif in the Ig-like domain of human and murine Fcα/μR that is conserved in the first Ig-like domain of human, murine, and rat poly-IgR (Fig. 3A [11]). Previous reports suggested that this motif is important for IgM and IgA bindings to the poly-IgR [16,17]. Thus, this region is predicted to be also important for IgA and IgM bindings to the Fcα/μR. To evaluate this possibility, we synthesized the peptides corresponding to this motif sequence (motif peptide) (Fig. 3A) and to the chicken egg ovalbumin (OVA) as a control for competitive binding analyses. Each anti-mFcα/μR mAb was preincubated with the motif or control peptide and then used for staining of the BW5147 transfectant expressing mFcα/μR. As demonstrated in Fig. 3B and Table 1, pre-incubation of the mAbs in the groups I, III, and IV with the motif peptide significantly decreased the antibody binding to the transfectant. In contrast, the binding of any of the mAbs in the groups II and V to the transfectant was not affected by preincubation with the motif peptide. These results suggested that the mAbs in the groups I, III, and IV recognize epitope in the motif peptide in the Ig-like domain of Fcα/μR.

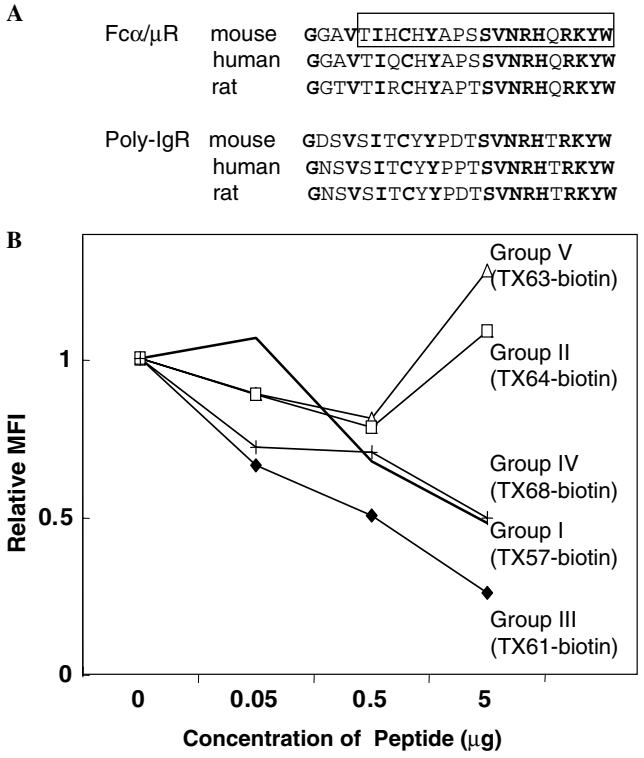

Fig. 3. Competitive binding analyses between mFcα/μR mAbs and a peptide from the conserved amino acids sequence. (A) The conserved amino acid sequences in the Ig-like domain of Fcα/μR and in the first Ig-like domain of poly-IgR are shown in each species indicated. The peptide in the box was used for the competitive binding analyses for (B). (B) Biotin-conjugated anti-mFcα/μR mAb indicated was incubated with the peptide derived from the conserved amino acid sequences, as shown in box (A), or OVA peptide at variable concentrations indicated. BW5147 transfectant expressing mFcα/μR was stained with the antibody-peptide mixtures, followed by FITC-conjugated streptavidin and analyzed by flow cytometry. Data are shown in relative MFI of the transfectant stained with antibody-motif peptide mixtures, as compared with that stained with antibody-control peptide (OVA) mixtures. Data are representative from more than three independent experiments.

Although the mAbs in the groups I, III, and IV block the IgA and IgM bindings to mFcα/μR as a result of competitive binding to the motif peptide which is also conserved in human Fcα/μR, only the mAbs in group III cross-reacted to human Fcα/μR (Table 1). There is one amino acid difference between the motif peptide sequences of mouse and human Fcα/μR (Fig. 3A), which might be involved in epitope recognized by the mAbs in groups I and IV. It should be noted that, while the motif peptide is also highly conserved in murine, human, and rat poly-IgR (Fig. 3A), no mAbs in the group I to V cross-reacted to the murine poly-IgR (data not shown).

In conclusion, by using mice deficient in *Fcα/μR* gene, we have successfully developed anti-mFcα/μR mAbs recognizing epitopes within the highly conserved peptide in murine, human, and rat Fcα/μR. These mAbs efficiently block the IgA and IgM bindings to mFcα/μR, suggesting the presence of IgA and IgM binding site in the peptide sequence of Fcα/μR as well as poly-IgR. These mAbs

should be helpful for molecular and functional characterization of IgA and IgM interaction with Fcα/μR.

Acknowledgments

We thank Y. Soeda for secretarial assistance. This research was supported in part by the grants provided by the Ministry of Education, Science and Culture of Japan and Special Coordination Funds of the Science and Technology Agency of the Japanese Government.

References

[1] M. Daeron, Fc receptor biology, Annu. Rev. Immunol. 15 (1997) 203–234.
[2] J.V. Ravetch, R.A. Clynes, Divergent roles for Fc receptors and complement in vivo, Annu. Rev. Immunol. 16 (1998) 421–432.
[3] J.V. Ravetch, S. Bolland, IgG Fc receptors, Annu. Rev. Immunol. 19 (2001) 275–290.
[4] T. Takai, Roles of Fc receptors in autoimmunity, Nat. Rev. Immunol. 2 (2002) 580–592.
[5] C.A. Whelan, A functional role for Fc mu receptors on human lymphocytes, Immunol. Lett. 3 (1981) 249–254.
[6] L. Ercolani, A. Novogrodsky, M. Suthanthiran, A.L. Rubin, K.H. Stenzel, Expression of Fc mu receptors by human T lymphocytes: effects of enzymes, metabolic inhibitors, and X-irradiation, J. Immunol. 127 (1981) 2044–2051.
[7] A. Mathur, R.G. Lynch, G. Kohler, Expression, distribution and specificity of Fc receptors for IgM on murine B cells, J. Immunol. 141 (1988) 1855–1862.
[8] T. Ohno, H. Kubagawa, S.K. Sanders, M.D. Cooper, Biochemical nature of an Fc mu receptor on human B-lineage cells, J. Exp. Med. 172 (1990) 1165–1175.
[9] L. Pricop, H. Rabinowich, P.A. Morel, A. Sulica, T.L. Whiteside, R.B. Herberman, Characterization of the Fc mu receptor on human natural killer cells. Interaction with its physiologic ligand, human normal IgM, specificity of binding, and functional effects, J. Immunol. 151 (1993) 3018–3029.
[10] T. Nakamura, H. Kubagawa, T. Ohno, M.D. Cooper, Characterization of an IgM Fc-binding receptor on human T cells, J. Immunol. 151 (1993) 6933–6941.
[11] A. Shibuya, N. Sakamoto, Y. Shimizu, K. Shibuya, M. Osawa, T. Hiroyama, H.J. Eyre, G.R. Sutherland, Y. Endo, T. Fujita, T. Miyabayashi, S. Sakano, T. Tsuji, E. Nakayama, J.H. Phillips, L.L. Lanier, H. Nakauchi, Fc alpha/mu receptor mediates endocytosis of IgM-coated microbes, Nat. Immunol. 1 (2000) 441–446.
[12] N. Sakamoto, K. Shibuya, Y. Shimizu, K. Yotsumoto, T. Miyabayashi, S. Sakano, T. Tsuji, E. Nakayama, H. Nakauchi, A. Shibuya, A novel Fc receptor for IgA and IgM is expressed on both hematopoietic and non-hematopoietic tissues, Eur. J. Immunol. 31 (2001) 1310–1316.
[13] J.P. Kinet, P. Launay, Fc alpha/microR: single member or first born in the family? Nat. Immunol. 1 (2000) 371–372.
[14] K.E. Mostov, Transepithelial transport of immunoglobulins, Annu. Rev. Immunol. 12 (1994) 63–84.
[15] Y. Shimizu, S. Honda, K. Yotsumoto, S. Tahara-Hanaoka, H.J. Eyre, G.R. Sutherland, Y. Endo, K. Shibuya, A. Koyama, H. Nakauchi, A. Shibuya, Fc(alpha)/mu receptor is a single gene-family member closely related to polymeric immunoglobulin receptor encoded on Chromosome 1, Immunogenetics 53 (2001) 709–711.
[16] M.A. Bakos, A. Kurosky, R.M. Goldblum, Characterization of a critical binding site for human polymeric Ig on secretory component, J. Immunol. 147 (1991) 3419–3426.
[17] M.A. Bakos, A. Kurosky, C.S. Woodard, R.M. Denney, R.M. Goldblum, Probing the topography of free and polymeric Ig-bound human secretory component with monoclonal antibodies, J. Immunol. 146 (1991) 162–168.