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Characterization of anti-channel catfish MHC class IIβ monoclonal antibodies

Mohadetheh Moulana [a], Jason Evenhuis [b], Mark Albertino [c], Ulla Godwin [c], Evgueni I. Kountikov [a], Tor B. Stuge [a], Melanie Wilson [a], Eva Bengtén [a,*], Norman W. Miller [a], Thomas J. McConnell [c]

[a] Department of Microbiology, University of Mississippi Medical Center, 2500 North State Street, Jackson, MS 39216, USA
[b] USDA/ARS National Center for Cool and Cold Water Aquaculture, 11861 Leetown Road, Kearneysville, WV 25430, USA
[c] Department of Biology, East Carolina University, N108 Howell Science Complex, 1000 East 10th Street, Greenville, NC 27858, USA

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Abstract

This study characterizes four monoclonal antibodies (mAb) developed against the major histocompatibility complex (MHC) class II β chain of the channel catfish, *Ictalurus punctatus*. Immunoprecipitations using catfish clonal B cells revealed that each of these mAbs immunoselected proteins of approximately 32 and 36 kD, which are of the appropriate sizes for MHC class II α and β chains, respectively. Cell distribution studies using a fluorescence-activated cell sorter (FACS) combined with RT-PCR analyses demonstrated that MHC class II β is expressed at a high density on catfish clonal macrophage, B and T cell lines, on alloantigen stimulated leukocytes, and on lipopolysaccharide-induced B-cell blasts. Collectively, these results demonstrate the potential importance of these antibodies as reagents in future studies dealing with the functional role of MHC class II molecules in immune recognition of self from non-self.
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Keywords: Major histocompatibility complex; Monoclonal antibody; Peripheral blood leukocytes

1. Introduction

Mammalian MHC molecules play a central role in the immune recognition of non-self. For example, MHC class I molecules are widely expressed on all nucleated cells and present processed peptides from endogenously produced antigens to CD8 positive T cells. In contrast, MHC class II molecules present peptides from exogenously produced antigens to CD4 positive cells, and for the most part are restricted to professional antigen presenting cells (APC), which include the monocyte/macrophage, dendritic and B cell lineages. However, MHC class II can be expressed on human activated T cells (Margulies and McCluskey, 2003). Early studies, based on analogy to mammalian immune responses, demonstrating the ability of teleosts to reject allografts (Hildemann, 1970), respond in mixed leukocyte reactions (Miller et al., 1986) and process and present exogenous antigens (Vallejo et al., 1991; Vallejo et al., 1992) suggested that MHC-like molecules are present in teleost species and function in a similar manner to their mammalian counterparts. In support of this contention, genes homologous to MHC class I, class II and B_2-microglobulin were subsequently

* Corresponding author. Tel.: +1 601 984 1739.
E-mail address: ebengten@microbio.umsmed.edu (E. Bengtén).

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described in a variety of different teleosts (Dixon et al., 1995; Bingulac-Popovic et al., 1997; Bartl, 1998, and reviewed in McConnell et al., 1998; Miller and Withler, 1998; and Stet et al., 2003), including the channel catfish (Godwin et al., 1997; Criscitiello et al., 1998; Antao et al., 1999; Godwin et al., 2000; Antao et al., 2001; Quiniou et al., 2005). Furthermore, the expression of both CD4 and CD8 genes has also been described in several teleost species (Hansen and Strassburger, 2000; Moore et al., 2005; Bernard et al., 2006; Buonocore et al., 2006; Dijkstra et al., 2006; Laing et al., 2006; Edholm et al., 2007; Picchietti et al., 2008). Together, these findings suggested that teleost adaptive immune systems use an antigen recognition framework similar to that found in mammalian systems.

However, there is still little direct experimental evidence concerning either the cellular expression of classical and non-classical teleost MHC class I and class II molecules, or their function in antigen recognition by fish T helper, T cytotoxic or NK cells. This has largely been due to the lack of appropriate antibody reagents that define various teleost leukocyte subpopulations and MHC molecules. Recently though, an anti-rainbow trout MHC class I monoclonal antibody (mAb; (Dijkstra et al., 2003; Fischer et al., 2005) and anti-catfish MHC class II α and β mAbs have been described (Fuller et al., 2004; Thankappan et al., 2006). Here, we characterize four of the anti-catfish MHC class II β mAbs and provide novel information concerning the cellular expression of teleost MHC class II antigens.

2. Materials and methods

2.1. Experimental animals, cell lines and cell cultures

Channel catfish (1–2 kg) were obtained from a commercial source (ConAgra, Isola, MS) and maintained in individual tanks as described previously (van Ginkel et al., 1992). Leukocytes from catfish peripheral blood (PBL), spleen, pronephros, and mesonephros were isolated as previously described (van Ginkel et al., 1992; Miller et al., 1994a). Freshly isolated catfish leukocytes and cell lines were cultured at 27 °C in catfish AL-medium, which consists of equal parts AIM-V and L-15 (Invitrogen Life Technologies) adjusted to catfish tonicity with 10% (v/v) deionized water and supplemented with 2–4% heat inactivated pooled catfish serum, 1 mg/ml $NaHCO_3$, 50 units/ml penicillin, 50 μg/ml streptomycin, 20 μg/ml gentamicin and 50 μM 2-mercaptoethanol (Miller et al., 1994a). 1G8 and 3B11 are cloned B cell lines, 28S.3 is a cloned T cell line and 42TA is a macrophage line, all were derived from the PBL of different outbred catfish (Miller et al., 1994a, 1994b; Wilson et al., 1998). The clonal T cell line G14D (Hogan et al., 1999) was derived from a homozygous gynogenetic catfish (Goudie et al., 1995). G5F is a fibroblast cell line, which was derived from fin snips from a different gynogenetic catfish, and was provided by Dr. V.G. Chinchar, UMC. TS32.15 and TS32.17 are clonal non-autonomous antigen-dependent cytotoxic T cell lines (CTL) developed from the same alloantigen immunized outbred fish. They require weekly restimulation with irradiated allogeneic cells for their continued proliferation (Stuge et al., 2000).

The alloantigen-dependent CTL (5×10^5) were stimulated with 2×10^6 irradiated (2500–4000 rad) allogeneic 3B11 B cells and cultured in 1 ml AL-5 media per well in 24-well plates. Five days after stimulation the cells were harvested for RNA preparation and flow cytometry analyses. At this time point, as assessed by RT-PCR, allogeneic 3B11 cells were no longer present. Catfish PBL were stimulated with mitogens as described previously (Miller et al., 1994a). Typically, freshly isolated PBL (1×10^6) were cultured in 24-well plates (Corning Inc., Corning, NY) in AL-5 media with either 50 μg ConA (Sigma Chemical Co.), 100 μg/ml of LPS (from *Salmonella typhimurium*, Sigma) or 0.05 μg/ml PMA plus 0.5 μg/ml calcium ionophore (A23187). Proliferating cells were harvested on day 4 or 5, as indicated, for RNA preparation and flow cytometry analyses. A day 0 sample was also harvested from unstimulated PBL. Mixed leukocyte cultures (MLC) were generated by stimulating freshly isolated PBL (1×10^6) with irradiated allogeneic 3B11 B cells (2×10^6) according to the protocol described previously (Stuge et al., 1997). The stimulated cells were harvested on day 4 or 5 for RNA preparation and flow cytometry analyses. After Lymphoprep isolation, tissue leukocytes were washed in ice-cold catfish RPMI-1640 (Gibco, BRL) and used for RNA preparation and flow cytometry analyses.

2.2. MHC class II β mAbs

The anti-catfish MHC class II β chain (Bs1.1, Bs3.1, Bs4.1 and Bs6.1) mAbs were developed using standard protocols (Harlow and Lane, 1988). Briefly, MHC class II β recombinant (r) protein was produced in *E. coli* by directional cloning of the β domains into the pQE-30 expression vector (Qiagen). Primers used in the cloning included either a BamHI or HindIII site (underlined in Table 1) and corresponded to amino acid sequences at the beginning of β1, NFLSQPD and at the end of β2,

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Table 1
Channel catfish gene-specific primers used for cloning and RT-PCR

Target	Sequence 5′–3′	GenBank accession #
TM 335 F[a]	5′-GCA*GGATCC*AATTTCCTGTCACAGCCAGAT-3′	U77598
TM 333 R[a]	5′-GTG*AAGCTT*TCACTTACTCTTATCAGGTTCAGG-3′	U77598
MHCIIB F[b]	5′-CAGGTTAAAGTGAAGTTGGTT-3′	U77598
MHCIIB R[b]	5′-CCACTTATAGTTCATGGGTTTG-3′	U77598
EF1α F[b]	5′-GACTGCCACACTGCTCACATTG-3′	ABC75588
EF1α R[b]	5′-TTAGTTACTCAGCAGCTTTCTTCC-3′	ABC75588

[a] Primer used for cloning into expression vector.
[b] Primer used for RT-PCR.

MNYKW. The rMHC class II β plasmid was transformed into *E. coli* strain M15 [pREP4] and large-scale expression cultures were grown and purified according to the manufacturer's protocol. Balb/c mice were immunized with recombinant protein and mAbs were produced as previously described (Fuller et al., 2004). Monoclonal antibodies Bs1.1 and Bs4.1 and two other anti-catfish MHC class II mAbs Bc1.1 and Bc2.1, which work in Western blot analyses have been described previously (Chen et al., 1984; Fuller et al., 2004; Thankappan et al., 2006).

2.3. RT-PCR

Total RNA from various catfish cell lines was prepared using RNA-Bee (TEL-TEST, INC); and treated with DNAse I (Invitrogen Life Technologies) according to the manufacturer's recommended protocol. One μg of RNA was reverse transcribed using an oligo-T primer and 200 units of Superscript III reverse transcriptase (Invitrogen Life Technologies). RT-PCR was performed using specific primers for MHC class II β and the housekeeping gene elongation factor-1 alpha (EF1α; Table 1) as a control. Program parameters were: 2 min 94 °C, followed by 29 cycles of 94 °C 30 s, 58 °C 30 s, 72 °C 1 min, then final extension at 72 °C for 10 min. PCR products were visualized on 10% Tris-acetate-EDTA agarose gels, cloned into pCR4TOPO (Invitrogen Life Technologies) and verified by sequencing.

2.4. Solid phase immunoselection technique (SPIT)

SPIT assays were performed as previously described (Chen et al., 1984; Rycyzyn et al., 1996). Briefly, 96-well flat bottom ELISA plates (Corning) were coated overnight with goat anti-mouse IgG (Southern Biotech) in carbonate buffer, pH 9.6. Wells were washed with Tris-buffered saline containing 0.05% Tween 20 (TBST) and other binding sites were blocked with 1% BSA in TBST for 1 h at 37 °C. The wells were then incubated with hybridoma culture supernatants containing either anti-catfish MHC class II β mAb, anti-rainbow trout Igμ mAb (1.14, negative control; (DeLuca et al., 1983) or anti-catfish Igμ mAb (9E1, positive control; (Miller et al., 1987) for 1 h at 37 °C. Approximately 3×10^7 1G8 cells were surface biotinylated (Meier et al., 1992) and lysed in 200 ml of 10 mM Tris-HCL, pH 7.3, 150 mM NaCl, 0.5% NP-40, 1 mM PMSF, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 5 μg/ml pepstatin A. Nuclear and cell debris were removed from lysates by centrifugation (11,000 × g, 10 min at 4 °C). Wells were incubated with 100 μl of cell lysates for 4 h at 4C and after washing with lysis buffer the bound proteins were dissociated with 40 μl reducing Laemmli's sample buffer. Immunoselected proteins were analyzed using 10% SDS-PAGE. After electrophoresis, proteins were transferred to nitrocellulose membranes (Amersham Pharmacia Biotech), the membranes were blocked with 3% BSA in TBST, and the proteins were visualized using strepavidin-conjugated horseradish peroxidase (Southern Biotech) and the enhanced chemiluminescence detection system (Amersham Pharmacia Biotech).

2.5. Flow cytometry

Cells (5×10^5 to 1×10^6) were harvested and washed with ice-cold RPMI 1640 medium and 100 μl of the cell suspension was incubated with 100 μl of either anti-catfish MHC class II β mAbs, anti-catfish neutrophil marker (51A), anti-catfish Igμ mAb (9E1, positive control), anti-rainbow trout Igμ mAb (1.14, negative control) for 45 min on ice. Dual staining experiments were performed using mAb Bs1.1 in combinations with one of the following mAbs Bs3.1, Bs4.1, Bs6.1, 9E1, 1.14, or 4–20, which reacts with catfish thrombocytes (Passer et al., 1997; Table 2). After washing, cells were incubated with 50 μl of phycoerythrin (PE)- or fluorescein isothiocynate (FITC)-conjugated goat anti-mouse isotype secondary antibodies (Southern Biotech)

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Table 2
Antibodies used in characterization of catfish leukocytes

Designation	Isotype	Marker	Cell type	Use[a]	Reference
9E1	IgG1	Catfish IgM	B cells, FcR bearing cells	F, S	Miller et al., 1987
1.14	IgG1	Trout IgM	B cells	F, S	DeLuca et al., 1983
4–20	IgG1	Integrin subunits	Thrombocytes	F	Passer et al., 1997
51A	IgG1	Leucine zipper containing protein	Neutrophils	F	Ainsworth et al., 1990; Xue et al., 1999
MHC II Bs 1.1	IgG2a	MHC II beta	B cells, MØ, some T cells	F, S	Fuller et al., 2004
MHC II Bs 3.1	IgG1	MHC II beta	B cells, MØ, some T cells	F, S	This study
MHC II Bs 4.1	IgG1	MHC II beta	B cells, MØ, some T cells	F, S	Fuller et al., 2004
MHC II Bs 6.1	IgG1	MHC II beta	B cells, MØ, some T cells	F, S	This study

[a] F, flow cytometry; S, spit assay.

diluted 1:200 and 1:40, respectively. Samples were analyzed for either single or two-color staining using a FACScan flow cytometer (Becton Dickinson).

3. Results and discussion

3.1. Anti-catfish MHC class II β mAbs immunoprecipitate MHC class II αβ heterodimers

The anti-catfish MHC class II β mAbs, Bs1.1 (IgG2a kappa), Bs3.1 (IgG1 kappa), Bs4.1 (IgG1 kappa), and Bs6.1 (IgG1 kappa) were selected initially by ELISA screening using the rMHC class II β as antigen and then by flow cytometry using catfish PBL (see Table 2). None of these mAbs reacted with catfish MHC class II β chains by Western blot analysis, indicating that they react with the native form of the MHC class II β chain. Immunoprecipitation analyses using lysates from surface-biotinylated catfish 1G8 B cells revealed that each of the four mAbs immunoselected proteins of ~32 and ~36 kD, which were of the appropriate size for MHC class II molecules (Fig. 1). Previously, immunoprecipitation studies using allo-antisera developed against catfish monocyte cell cultures detected three major proteins, which had molecular weights similar to mammalian MHC proteins. A 43-kD band was considered to be the MHC class I α chain candidate, and a doublet band of 32–35 kD was predicted to be the MHC class II α and β chains (Vallejo et al., 1992). More recently, these conclusions were supported by immunoprecipitations and Western blots of lysates from unlabeled catfish 28S.3 T cells (Fuller et al., 2004). In that study, the 28S.3 cell lysates were immunoprecipitated with anti-MHC class II β mAbs Bs1.1 and Bs4.1 and the resulting proteins were then subjected to Western blotting with the anti-MHC class II β Bc1.1 mAb. In both cases a ~36 kD reactive protein was detected. Comparatively, Western blots of 28S.3 T cells using the anti-MHC class II α A3 mAb showed a ~32 kD protein, which is the predicted size of catfish MHC class II α. These previously described mAbs, A3 and Bc1.1, do not stain cell surfaces and react only with denatured MHC class II α and β, respectively. This in combination with our data imply that the four anti-MHC class II β mAbs tested here were able to immunoselect MHC class II molecules consisting of non-covalently associated MHC class II α (~32 kD) and β chains (~36 kD). Since no contaminating protein bands were found, it was concluded that these anti-MHC class II β mAbs were specific.

3.2. Cell surface expression of MHC class II β on catfish clonal leukocyte lines

As assessed by flow cytometry, all catfish clonal leukocyte cell lines tested showed positive staining with

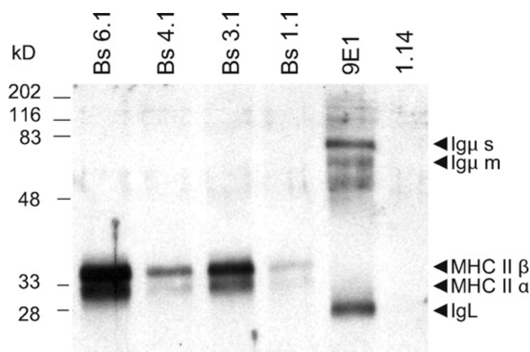

Fig. 1. Immunoprecipitation of surface-biotinylated catfish clonal 1G8 B cells using anti-catfish MHC class II β mAbs. Samples were electrophoresed on 10% SDS-PAGE under reducing conditions. Lanes are labeled according to anti-MHC class II β mAb used. Anti-catfish Igμ (9E1) and anti-trout Igμ (1.14) were used as positive and negative controls, respectively. The detected IgM secreted form (s; ~70 kD) is larger than the membrane form (m; ~ 67 kD (Wilson et al., 1990)) and is likely the result of serum contamination since the culture media supplemented with catfish serum. Arrowheads identify the respective immunoselected proteins. Molecular weight markers are at left. Data shown is representative of three independent immunoprecipitations.

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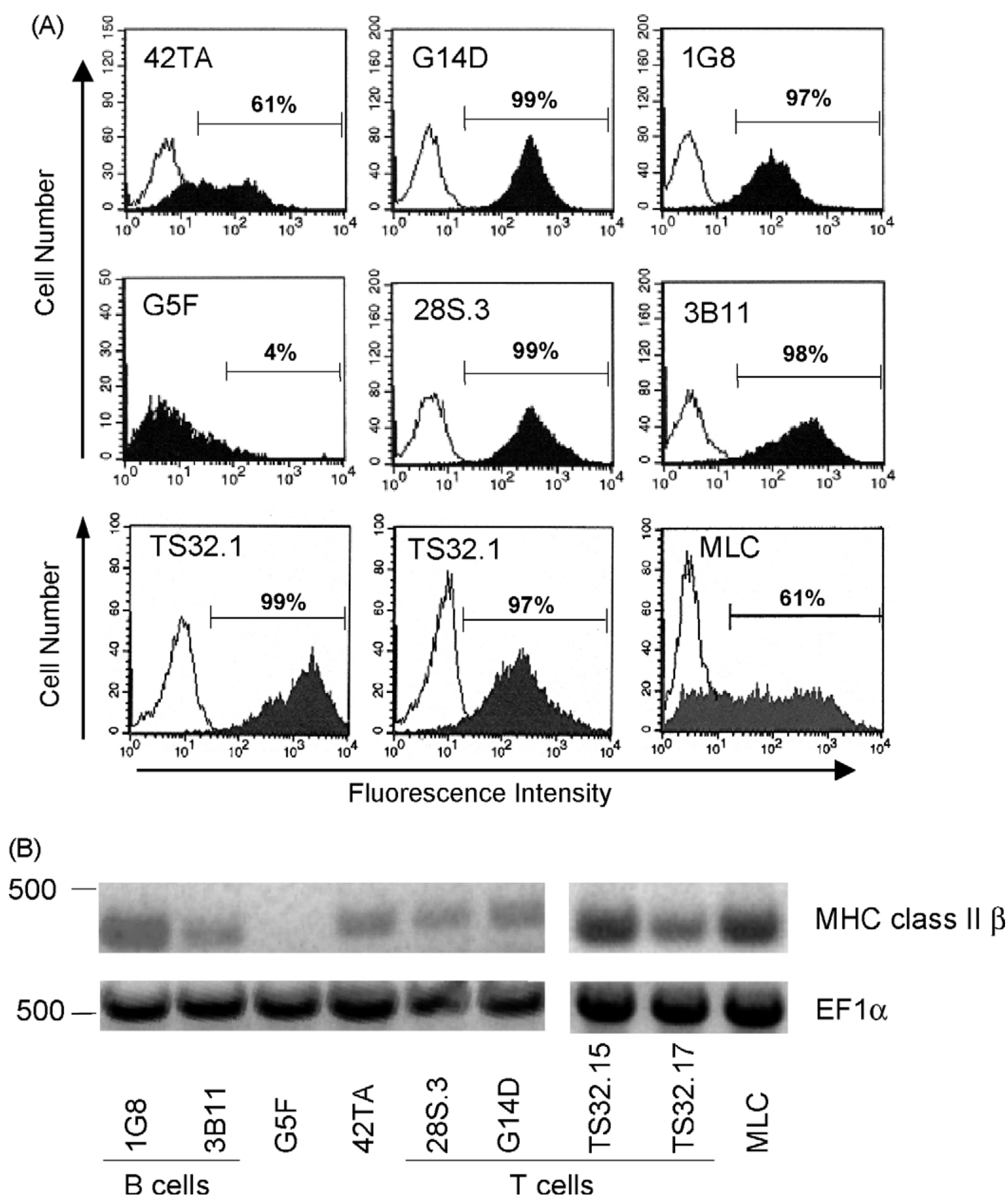

Fig. 2. Catfish clonal leukocyte lines differ in MHC class II β expression. (A) Cells assessed by flow cytometry using mAb Bs1.1 (black) as the representative anti-MHC class II β antibody or anti-trout Igμ 1.14 mAb as an isotype control (unshaded) are shown. Histograms are labeled according to cell type. Autonomous cell lines: 42TA macrophages; G14D and 28S.3 T cells; 1G8 and 3B11 B cells; G5F fibroblasts. Non-autonomous CTL cell lines: TS32.15 and TS32.17. The MLC is from a 4-day culture. Bars mark the percentage of MHC class II β stained cells. (B) RT-PCR analyses of MHC class II β and EF1α (positive control) are also shown. Band sizes are at left and PCR products were verified by sequencing.

the four anti-MHC class II β mAbs when compared to the negative control mAb 1.14 (anti-rainbow trout Igμ, Fig. 2A). Additionally, RT-PCR analysis confirmed that the cells were expressing MHC class II β message (Fig. 2B). In contrast, G5F fibroblast cells were negative for MHC class II β expression as assessed by both flow cytometry and RT-PCR analyses. The MHC class II β surface and message expression by catfish T cell lines G14D, 28S.3, TS32.15 and TS32.17 is consistent with the expression of MHC class II molecules by activated T cells in humans and mice (Margulies and McCluskey, 2003). MHC class II molecules have also been shown to be expressed on adult *Xenopus* T cells (Flajnik et al., 1990) and as well as on dog, cat, and horse T lymphocytes (Alejandro et al., 1984; Crepaldi et al., 1986; Neefjes et al., 1986). Such findings indicate that T

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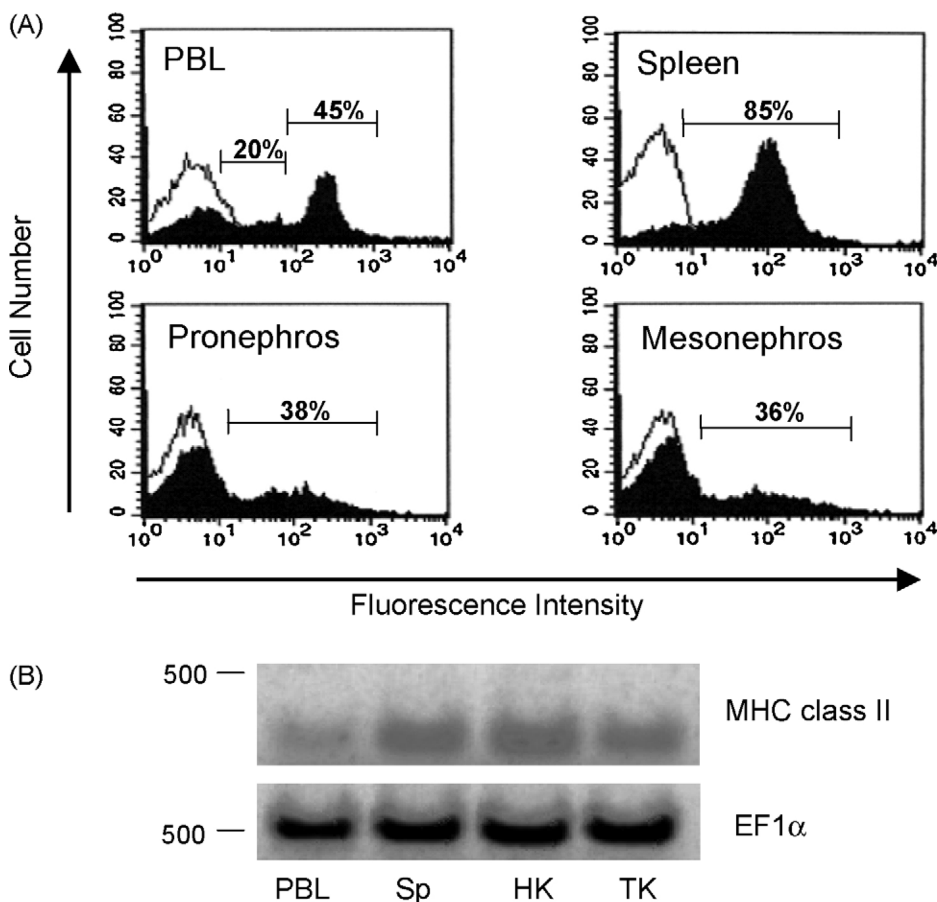

Fig. 3. Catfish tissue leukocytes express MHC class II β. (A) Tissue leukocytes assessed by flow cytometry using mAb Bs1.1 (black) as the representative anti-MHC class II β antibody or anti-trout Igμ 1.14 mAb as an isotype control (unshaded) are shown. Histograms are labeled according to the tissue type examined: PBL; spleen (Sp); pronephros (HK); mesonephros (TK). Bars indicate the percentage of MHC class II β stained cells. (B) RT-PCR analysis of MHC class II β and EF1α (positive control) is shown below. Band sizes are at left. Data shown is from one representative catfish out of three.

cells in these animals, including the catfish, may act as antigen presenting cells. Expression of MHC class II β was also seen in cells from a MLC (Fig. 2), which does not contain B cells but does contain cytotoxic T cells and NK-like cells (Stuge et al., 2000).

As expected, the representative anti-MHC class II β mAb Bs1.1 reacted with PBL and cells from all lymphoid tissues tested (Fig. 3A). Also MHC class II β message was readily detectable in all of the tissues by RT-PCR (Fig. 3B). These results confirm and extend previous studies where MHC class II message was found expressed in various teleost lymphoid and myeloid tissues (Hansen et al., 1999; Koppang et al., 2003; Cuesta et al., 2006). Flow cytometric analyses of catfish PBL stained with anti-MHC class II β mAb Bs1.1 indicated three distinct populations; i.e., high intensity staining, moderate intensity staining and low to no staining (Fig. 3A). In comparison, the 85% of the cells isolated from spleen were MHC class II β positive, while cells from pronephros and mesonephros contained fewer numbers of MHC class II β expressing cells. The finding that all four of the anti-MHC class II β mAbs reacted with leukocytes from PBL and lymphoid tissues from each catfish tested indicates that these mAbs are pan specific. In this context, it should be noted that previous RFLP analyses using DNA from primary cells and cell lines derived from outbred animals have shown that the catfish MHC class II β locus is very polymorphic (Zhou, 2001; Godwin et al., 1997; Quiniou et al., 2005, and unpublished data). However, leukocytes from more than 50 outbred fish have been tested for reactivity with the anti-MHC class II β mAbs, and in no case was negative staining observed (data not shown). Thus, these observations further support the contention that these mAbs are pan specific rather than allele specific.

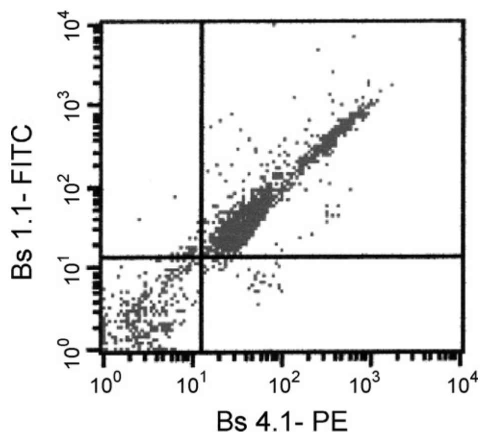

Fig. 4. Two-color flow cytometric analysis with Bs1.1 and Bs4.1. Freshly isolated PBL were first stained with mAb Bs1.1, then with mAb Bs4.1, followed by FITC and PE isotype specific secondary antibodies. Data shown is representative of three independent experiments.

3.3. Identification of cell phenotypes expressing MHC class II β in PBL

When anti-MHC class II β chain mAbs Bs1.1 (IgG2a) and Bs4.1 (IgG1) were used in two-color flow cytometry analysis both stained exactly the same cells with similar staining intensity profiles (Fig. 4). This finding demonstrated not only that these two mAbs recognized the same surface MHC class II β molecules on the same cells, but also recognized different epitopes within the MHC class II β chain, since competitive inhibition of binding was not observed. Also the moderate and high intensity MHC class II β populations could be readily observed. Exactly the same results were found when mAbs Bs3.1, and Bs6.1 were compared in two color flow cytometry analyses with Bs1.1 (data not shown). Two color flow cytometry analyses could not be performed comparing Bs3.1, Bs4.1 and Bs6.1 to each other because they were all of same antibody isotype (IgG1).

Two color flow cytometry analyses were also used to help determine the phenotypes of the PBL showing these three different staining intensities for MHC class II β. The three mAbs used were: 9E1, specific for catfish Igμ; 4–20, specific for an integrin found on catfish thrombocytes; and 51A, specific for catfish neutrophils. Staining of PBL with both Bs1.1 and 9E1 showed that the cells with highest MHC class II β staining were also positive for surface IgM and are most likely B cells (Fig. 5). In addition, a small population of IgM positive cells expressed low to no membrane staining for MHC class II β. Here it should be noted that some catfish NK-like cells have been shown to be pre-armed with IgM via a putative FcR for Igμ (Shen et al., 2003). Furthermore, the majority of MHC class II β expressing cells (58%) do not express surface IgM. Catfish leukocytes that are IgM negative and express moderate levels of MHC class II β staining are positive for an integrin found on catfish thrombocytes (mAb 4–20). These results imply that at least some catfish thrombocytes express MHC class II. However, there is fish to fish variability in the detection of MHC class II β on catfish thrombocytes, i.e., 4–20 positive cells, as shown in (Fig. 5). It should be noted that the majority of the fish examined, like fish 1, have thrombocytes that are positive for MHC class II, and only rarely is a fish found to have MHC class II β negative thrombocytes as seen in fish 2. However, fish having MHC class II β negative thrombocytes have other leukocytes that react with the anti-MHC class II β mAbs. Thus, lack of thrombocyte reactivity is not due to the mAbs reacting with polymorphic determinants, but rather that the thrombocytes lack MHC class II molecules. A reason(s) for the absence of MHC class II positive thrombocytes in some fish is currently unknown.

In catfish, neutrophils are found in low numbers (1–5%) in normal PBL, but are dramatically increased in stressed fish (Ellsaesser and Clem, 1986). Catfish neutrophils are large granular cells that stain positive with mAb 51A (Ainsworth et al., 1990). Thus, in order to assess anti-MHC class II β staining on neutrophils, PBL from fish stressed by handling were stained with both 51A and Bs4.1 mAbs. As indicated by scatter analyses Bs4.1 failed to react with the large granular leukocyte population that was reactive with 51A (Fig. 6). Even though neutrophils are a major phagocytic cell type in catfish, this lack of MHC class II would suggest that they do not play a role in antigen presentation in the context of MHC class II. However, our findings do not rule out the possibility that MHC class II could be induced in neutrophils under certain conditions. In this context it has been shown that both human and murine neutrophils can express low levels of MHC class II under certain inflammatory conditions (Sandilands et al., 2005; Culshaw et al., 2008). In addition it has been shown that acidophilic granulocytes (defined by mAb G7) in seabass express message for MHC class II alpha, and thus have been implicated as antigen presenting cells (Cuesta et al., 2006). However, due to the lack of a cell marker for catfish acidophilic granulocytes it is not known if this cell type exists in catfish or if it expresses MHC class II molecules. Other leukocyte populations that are either negative or positive for MHC class II β are currently unknown

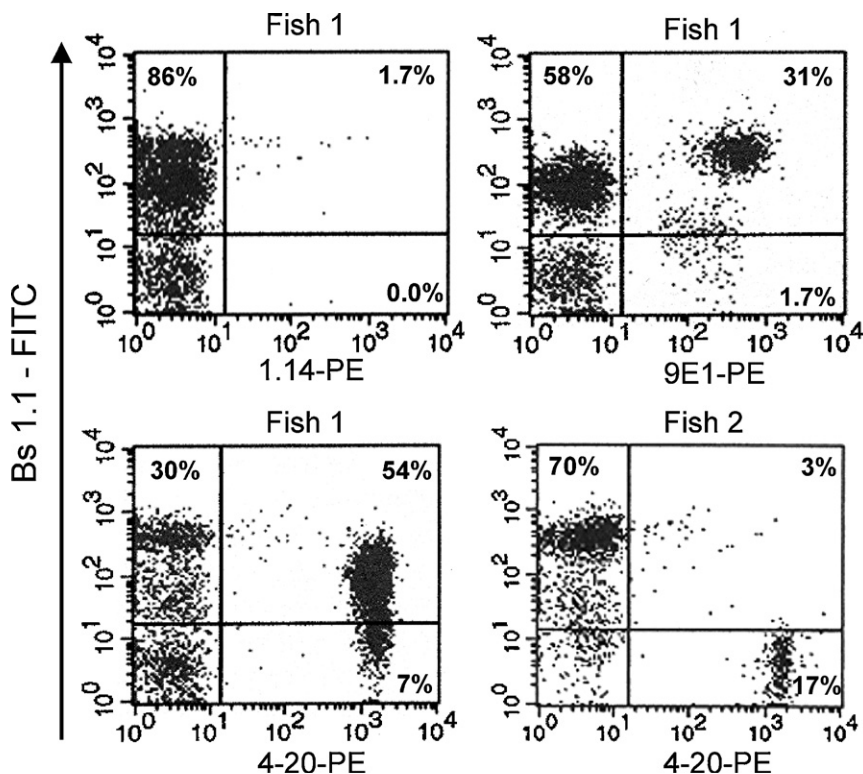

Fig. 5. Two-color flow cytometric analysis comparing expression of MHC class II β and other cell surface antigens on catfish PBL. Freshly isolated PBL were two-color-stained with anti-MHC class II β Bs1.1 and anti-catfish Igμ (9E1), anti-catfish thrombocyte (4–20), or anti-trout Igμ (1.14) as a negative control. Goat anti-mouse isotype specific secondary antibodies were conjugated to PE or to FITC. Data shown for fish 1 is representative of 8 out of 10 catfish and data for fish 2 is representative of 2 out of 10 catfish.

due to the lack of definitive antibody markers for most catfish leukocyte subpopulations.

3.4. Modulation of surface MHC class II β expression on PBL by mitogen stimulation

Stimulation of PBL with LPS, ConA, or a combination of phorbol ester and calcium ionophore (PMA/Ca^{2+}) resulted in blast transformation and increased cell numbers peaking by days 4–5 after stimulation (Miller et al., 1994a). Initial anti-MHC class II β staining of unstimulated PBL showed that 21% of the total cell population had high intensity MHC class II β staining with a geometric mean intensity of ∼223. LPS stimulated cells on day 4 had 46% of the total cells exhibiting high intensity MHC class II β staining with a geometric mean of ∼332. Comparatively, ConA stimulation did not result in a large increase in numbers

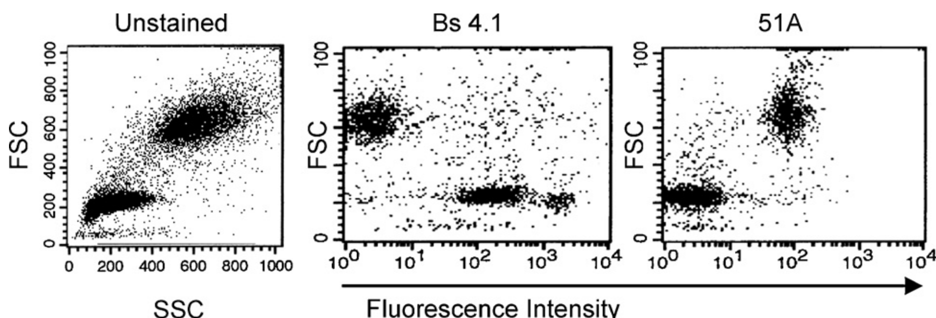

Fig. 6. Catfish neutrophils do not express MHC class II β. Freshly isolated catfish PBL from fish stressed by handling were stained with mAbs Bs4.1 or 51A and examined by flow cytometry. A dot plot of unstained PBL revealed a substantial population of large granular cells, i.e., neutrophils confirming that the fish was stressed. SSC, side scatter; FSC, Forward scatter. Data shown is one representative catfish out of five.

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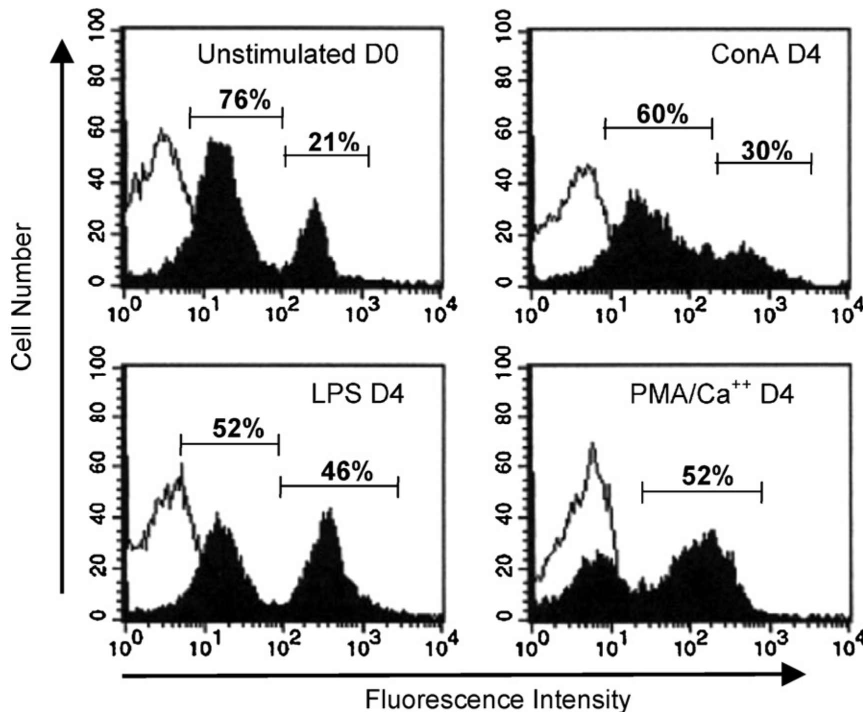

Fig. 7. Flow cytometric analysis comparing expression of MHC class II β on catfish mitogen stimulated PBL. MHC class II expression was assessed by flow cytometry using anti-MHC class II β Bs1.1 mAb (black) or anti-trout Igμ 1.14 mAb as an isotype control (unshaded). Histograms are labeled according to the mitogen used: Day 0 represents unstimulated freshly isolated PBL, the ConA, LPS and PMA/Ca^{2+} cultures were analyzed on day 4, which is the day when the largest cell number expansion occurs (Miller et al., 1994a). Data shown is from one representative catfish out of three.

of high intensity staining cells (30%) and only caused a slight increase in the geometric mean intensity to ~265. PMA/Ca^{2+} ionophore stimulation yielded an increase in staining with 52% of the cells staining with moderate intensity and a geometric mean intensity of ~164 (Fig. 7). The particular fish used to generate this data had a low percentage (~3–4%) of cells on day 0 that were negative for surface MHC class II β expression, however PMA/Ca^{2+} stimulation increased the number of MHC class II β negative cells to over 40%. This demonstrates that PMA/Ca^{2+} increases both MHC class II β positive and negative cell populations, however the cell types that make up the negative population have yet to be identified.

In summary, the four anti-MHC class II β mAbs described here were pan specific and able to immunoprecipitate MHC class II molecules from the cell surface of catfish B cells. The immunoprecipitated MHC molecules were composed of non-covalently associated MHC class II α chains of ~32 kD and β chains of ~36 kD. These antibodies also enabled analysis of MHC class II expressing leukocytes by flow cytometry, and demonstrated not only that catfish B cell and macrophage lines expressed MHC class II, but also catfish T cells. Double staining of catfish PBL with anti-MHC class II β and anti-IgM mAbs revealed that B cells express the highest levels of MHC class II. This finding has not been demonstrated previously and may be related to the recent finding that teleost B cells are potent and proficient phagocytic cells (Li et al., 2006). Although each of these mAbs recognized catfish MHC class II β they each recognized a unique epitope within the molecule. This study illustrates the potential importance of these antibodies as reagents in future studies dealing with the functional role of MHC class II molecules in immune recognition of self from non-self.

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