Characterization of Monoclonal Antibodies against Antigens Shared with Neutrophils and Macrophages in Rainbow Trout *Oncorhynchus mykiss*

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ABSTRACT — Four monoclonal antibodies (MAbs: Mm, MGm, MGcp1 and MGcp2) were produced against peritoneal exudate cells of rainbow trout, *Oncorhynchus mykiss*. The MAbs were characterized by immunofluorescence assay, flow cytometry, immunoelectron microscopy and Western blotting, and utilized to isolate macrophages and neutrophils by magnetic cell sorting. The immunofluorescence assay and flow cytometry showed that each of the MAbs was specific for both macrophage and neutrophil populations and these cells were observed in leucocytes from various origins, i.e. the head kidney, peripheral blood and peritoneal exudate. Mm and MGm reacted with membrane antigens, while MGcp1 and MGcp2 reacted with intracellular antigens. The MAb-positive cells were identified as both macrophages and neutrophils by electron microscopy using immunogold labelling. A Western blot analysis revealed that the MAbs detected different determinants (Mm; 205, 151 and 107 kDa; MGm; 205, 143, 107, 76, 55 and 52 kDa; MGcp1; no reaction; MGcp2; 64 kDa). Macrophages and neutrophils were efficiently isolated by a magnetic cell separation system with Mm and MGm. These antibodies will be useful for identifying and isolating rainbow trout macrophages and neutrophils, which facilitate studies of trout leucocytes.

Key word: MAb, rainbow trout, macrophage, neutrophil, *Oncorhynchus mykiss*

Monoclonal antibodies (MAbs) are useful tools in immunological research and have contributed to the identification and separation of leukocyte populations. B cells in fish, as in mammals, can be identified by MAbs that detect cell surface immunoglobulin. Such studies have been carried out in carp (Secombes *et al.*, 1983), rainbow trout (Thuvander *et al.*, 1990) and sea bass (dos Santos *et al.*, 1997). MAbs against other sub-populations of leukocytes have also been reported such as channel catfish T cells (Ainsworth *et al.*, 1990). Approximately 50 MAbs against fish leukocytes or immunoglobulin are listed by Romano (1998). In contrast to the mammalian CD (cluster of differentiation) system registering over 160 antigens recognized by MAbs (Kishimoto *et al.*, 1998), it is true that systematic editing of MAbs against fish leukocytes is delayed because of their less information. Therefore, further production and characterization of new MAbs against fish leukocytes would be helpful for developing fish immunological

studies.

Above all, MAbs against fish neutrophils and/or macrophages have potency as a useful tool because these cells play important roles in the immune response. Both neutrophils and macrophages are phagocytic cells, which migrate to inflammatory sites and eliminate microorganisms by phagocytosis (Ainsworth, 1992; Secombes and Fletcher, 1992). In addition, macrophages are involved in antigen presentation (Vallejo *et al.*, 1992) and production of cytokines (Secombes *et al.*, 1996). MAbs that react with these cell populations have been reported in channel catfish neutrophils (Bly *et al.*, 1990) and carp neutrophils and monocytes (Nakayasu *et al.*, 1998). To date, no MAb specific for rainbow trout macrophages and/or neutrophils has been established, although Slierendrecht *et al.* (1995) and Bowden *et al.* (1997) reported MAbs that reacted not only with these cell types but also with other leukocyte populations. MAbs specific for macrophages and neutrophils would allow isolation of the cells with high purity and no activation due to adherence to extracellular matrix, and visualize localizations of phagocytes during the

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process of inflammation or infection. Thus, following functional assay with the MAbs should help to elucidate the fish immune system involving phagocytes.

In this study, we attempted to produce MAbs that react with rainbow trout macrophages and/or neutrophils. Produced MAbs were characterized by flow cytometry, immunoelectron microscopy and Western blotting, and tested if they were applicable for magnetic cell sorting to isolate MAb labelled-macrophages and/or neutrophils.

Materials and Methods

Fish

Rainbow trout (*Oncorhynchus mykiss*), weighing 500–1000 g were obtained from the Yoshida Research and Training Station, Tokyo University of Fisheries. Fish were maintained at 16–18°C in outdoor concrete tanks and fed commercial pellets.

Leukocyte isolation

Leukocytes from three different origins, i.e. peritoneal exudate cells (PEC), head kidney leukocytes (HKL) and peripheral blood leukocytes (PBL), were collected and used for this study.

PEC were prepared for use as an immunogen against mice and for an analysis of the generated MAbs. They were prepared according to the method of Afonso *et al.* (1997) with some minor modifications. In brief, emulsified Freund's incomplete adjuvant (FIA; Difco) was injected into the peritoneal cavity of rainbow trout to elicit PEC at a rate of 2.0 mL per 500 g body weight. On the 10th day after the injection, the fish were killed by anaesthetic overdose in a tank containing 0.6 mL/L of ethylene glycol monophenyl ether (Wako) in water and exsanguinated from the caudal vein. Twenty mL of L-15 medium containing 5% FBS, 100 U/mL penicillin, 100 µg/mL streptomycin and 10 U/mL heparin was intraperitoneally (i.p.) injected. After the injection with 5 mL of air, the abdominal area was then massaged to suspend the PEC into the injected medium. The cell suspension was collected with a syringe fitted with an 18G needle. These steps were repeated one more time. The cell suspension was washed twice in the same medium by centrifugation at 400 g for 5 min at 4°C. Then, the cells were layered onto a 51% v/v Percoll (Amersham Pharmacia Biotech) and centrifuged at 400 × g for 25 min at 4°C. The harvested PEC were re-suspended in PBS(–) and kept on ice until use. A part of the PEC were stained by Giemsa's staining and Sudan Black B (SBB) staining to confirm the enrichment of macrophages and neutrophils.

HKL and PBL were prepared from unstimulated rainbow trout for analyses of the generated MAbs. Briefly, the head kidney was passed through a 50 µm stainless mesh with the same medium described above

and washed twice in PBS(–). Peripheral blood collected by a heparinized syringe was centrifuged to a pellet. The buffy-coat was collected and layered onto 54% v/v Percoll to separate erythrocytes from leukocytes. After centrifugation, cells banding at the interface were washed twice in PBS(–). HKL and PBL were kept on ice as well.

Production of monoclonal antibodies

Female BALB/c mice (4 weeks old) were i.p. injected with 1×10^7 trout PEC on days 0, 21 and 28. Three days before fusion on day 35, mice received an intravenous injection through a lateral tail vein. The spleen cells were fused with the P3X63-Ag.8.U1 myeloma cell line, according to the method of Köhler and Milstein (1975). Culture supernatants from the wells with growing colonies were screened by immunofluorescence assay (described below). After screening, four clones that reacted with trout macrophages and/or neutrophils were cloned by two times limiting dilution. The class and subclass of the monoclonal antibodies were determined by a sandwich ELISA using a mouse monoclonal antibody isotyping kit (Amersham Pharmacia Biotech).

Immunofluorescence assay (IFA)

The MAbs were screened by using ethanol-fixed PEC and PBL on 24-well slides. The slide preparations were incubated with hybridoma supernatants at 4°C for 1 h and washed twice in PBS before incubation with fluorescein isothiocyanate (FITC)-labelled goat anti-mouse Ig(G+A+M) F(ab')₂ fragment (Southern Biotechnology) at 4°C for 1 h. As a negative control, the samples were incubated with mouse anti-white spot syndrome virus MAb (Zhan *et al.*, 1999) as a primary antibody which was also used for the control in the following experiments. After washing the samples twice in PBS, they were observed with a fluorescence microscope (Olympus).

Flow cytometry (FCM)

MAbs were characterized by FCM using PEC, HKL, and PBL. Each cell suspension was centrifuged and adjusted to 2×10^6 cells/mL in fish Ringer's solution (0.75% NaCl, 0.02% KCl, 0.02% CaCl₂ and 0.002% NaHCO₃) containing 0.1% BSA (Sigma-Aldrich) and 0.1% sodium azide. To each 1-mL aliquot of cell suspension was added 100 µL of hybridoma supernatant and the mixture was incubated for 30 min on ice. After washing the cells twice in Ringer's solution, 50 µL of FITC-labelled anti-mouse Ig(G+A+M) F(ab')₂ was added to the cells and incubated for 30 min on ice. The cells were washed twice again and flow cytometric patterns were analyzed with an EPICS ELITE flow cytometer (Coulter).

Immunoelectron microscopy

The PEC were pelleted and then fixed at 4°C by the addition of 4% paraformaldehyde in 0.1M phosphate buffer. After 6 h of incubation, the cells were washed, dehydrated in graded ethanol concentrations and then embedded in LR-White resin (London Resin). Ultrathin sections were prepared and incubated in 5% BSA in PBS(–) containing 0.5% sodium azide for blocking at 4°C overnight. Sections on grids were layered onto the drop of each MAb and incubated for 1 h at room temperature (RT). After washing three times in PBS, they were incubated in a 1:100 dilution of 15-nm gold particle-conjugated goat anti-mouse IgG+IgM (British Biocell International) for 1 h at RT. They were washed three times in PBS and post-fixed with 2% glutaraldehyde in 0.1M phosphate buffer for 15 min at RT. After washing the sections three times in distilled water, they were stained with uranyl acetate followed by lead citrate and observed with a Hitachi H-7000 transmission electron microscope (Hitachi).

Western blotting

The PEC suspension (1×10^7 cells) in PBS(–) was centrifuged and the pelleted cells were lysed by addition of 100 μ L of lysis buffer (9.5M urea, 2% NP-40, 5% 2-mercaptoethanol, 500mM Tris-HCl, pH 6.8). The cell lysate was mixed with 100 μ L of SDS-denaturing buffer (5% SDS, 20% glycerol, 0.002% bromophenol blue) and boiled for 2 min (Laemmli, 1970). Aliquots of 15 μ L were subjected to a 11% SDS-PAGE gel and electrophoresed at 10 mA. After electrophoretic transfer onto a nitrocellulose membrane (Advantech) at 12 V for 90 min, immunoreactive bands were detected by combination of one of the MAbs and a peroxidase-conjugated sheep anti-mouse Ig (G+A+M) antibody (Dainippon Pharmaceutical) diluted 1:100 according to the manufacturer's recommendations. As a negative control, normal mouse serum was used for the primary antibody-reaction.

Magnetic cell sorting

After elimination of cell aggregation by passing the cells through a 50- μ m mesh, 1.5×10^8 cells of PEC were incubated with 1:20 diluted MAb in 10 mL of PBS (–) containing 0.5% BSA (PBSB) for 20 min at 4°C. The cells were washed twice in L-15 medium containing 5% FBS. Then the 1.5×10^8 cells were incubated with 500 μ L of 1:5 diluted magnetic bead-conjugated goat anti-mouse Ig antibody (Miltenyi Biotec) in PBSB for 20 min at 4°C according to the manufacturer's instructions. After washing the cells twice as above, the cells were suspended in degassed PBSB and applied to a magnetic separation column (Miltenyi Biotec) to which an external magnet was attached. The column was washed twice with degassed PBSB allowing MAb-negative cells to flow through. The MAb-positive cells left in

the column were collected following the removal of the column from the magnet. To separate the cells more accurately, the positive- and negative-cell fractions were re-applied to fresh magnetic separation columns. Smears of the collected MAb-positive cells, –negative cells, and the unseparated PEC were stained by Giemsa's staining and SBB staining to classify the leukocytes. Three thousand of cells from each fraction were counted for estimating a percentage of each cell type (magnification: $\times 400$).

Results

Production of monoclonal antibodies and screening

Of 384 wells, 346 (90.1%) had hybridoma colonies (an average of 3.2 colonies/well). The supernatants of 80 of these wells reacted with PEC in IFA. Of these supernatants, four did not react with erythrocytes, lymphocytes and thrombocytes using PBL samples. From colonies in these four wells, a targeted clone was cloned by two times limiting dilution. The four clones were designated Mm (Ig G3), MGm (Ig M), MGcp1 (Ig G1) and MGcp2 (Ig G1), respectively.

In IFA, the four clones specifically reacted with large cells that had an eccentric polymorphonucleus, vesicles in the cytoplasm and extended pseudopodia (Fig. 1(a)–(d)). These cells were thought to be monocytes/macrophages or neutrophils. Some of the large leukocytes showed no specific immunofluorescence. The control antibody didn't react with trout blood cells.

Flow cytometry

Cell types were classified by flow cytometry according to two major regions. One region (region A) showed low values in both forward scatter (FS) and side scatter (SS) and the other region (region B) showed high values in both parameters (Fig. 2 (a), (d), (g)). Cells in region A were considered as lymphocytes and thrombocytes, and those in region B were considered as granulocytes and monocytes/macrophages. Mm and MGm showed clear positive signals in each region B of the fluorescence histograms of PEC, HKL and PBL (Fig. 2 (c), (f), (i)). Few positive cells were observed in region A with either MAbs (Fig. 2 (b), (e), (h)). In the both analysis with Mm and with MGm, the percentages of the PEC, HKL and PBL that were positive in region B were 30–50%, 70–75% and 10–15%, respectively. None of the cells reacted with MGcp1, MGcp2 or the control antibody.

Immunoelectron microscopy

The MAb-positive cells were identified by immunoelectron microscopy, which showed that the four MAbs specifically reacted with macrophages and neutrophils. Macrophages were characterized by an eccentric nucleus with low condensed chromatin, many vesicles

Fig. 1. MAb-positive leukocytes visualized by immuno-fluorescence assay. **a.** Immunofluorescent micrograph with a MAb, Mm, showing that large cells with a lobulated nucleus are positive (arrows) and a small cell as a lymphocyte is negative (L). Some large cells that seem to be macrophages or neutrophils are negatively stained (arrowheads). Bar = 20 μ m. **b.** A MGm-positive monocyte (arrow) surrounded by small, negatively stained lymphocytes or thrombocytes. Bar = 10 μ m. **c.** Clusters of MGcp1-positive macrophages and neutrophils. Bar = 10 μ m. **d.** MGcp2-positive neutrophils (arrows) and -negative thrombocytes (Th). Erythrocytes (ER) are negatively stained. Bar = 20 μ m.

and microfilaments in the cytoplasm and well-extended pseudopodia. Neutrophils were characterized by an eccentric multilobed nucleus with condensed chromatin, and had fewer vesicles in the cytoplasm than macrophages. Lymphocytes were easily distinguished from macrophages and neutrophils by their small cell size, round shape and low N/C ratio. Thrombocytes were also easily distinguished by their small cell size, varying shape from round to spindle-shaped and canalicular system in cytoplasm. Only a few thrombocytes were observed in the PEC sections.

Macrophages and neutrophils were partly labelled with Mm- or MGm-gold particles. The particles were localized near the cell membrane and in the cytoplasm (Figs. 3–5). Most of the neutrophils were labelled by

the two MABs and contained more gold particles than did macrophages (not shown). With MGcp1 and MGcp2, gold particles were found in the cytoplasm of macrophages and neutrophils (Figs. 6 & 7). Macrophages or neutrophils with no gold particles were also observed in the sections tested with each MAB (figures not shown). There is no binding of gold particles to cells with the control antibody.

Western blotting

A Western blot analysis under reduced conditions was performed using the four MABs. Mm reacted with three bands of 205, 151, 107 kDa, MGm with six bands of 205, 143, 107, 76, 55 and 52 kDa, MGcp2 with one band of 64 kDa, while MGcp1 showed no reaction with

Fig. 2. Flowcytometric analysis of rainbow trout PEC, HKL and PBL with the MAbs, Mm and MGm. Flow cytometry FS/SS dot plots of PEC (a), HKL (d) and PBL (g) showing the region of lymphocytes and thrombocytes (region A) and the region of monocytes/macrophages and neutrophils (region B). Fluorescence histograms for Mm (black line) and MGm (gray line) were obtained from the cells in region A (b, e, h) and in region B (c, f, i). The two MAbs showed similar patterns. The MAb-positive cells were found in PEC, HKL and PBL, especially the cells in region B. Note that the two MAbs did not react with all of the cells in region B.

any bands (Fig. 8). No reaction by MGcp1 was probably due to denaturation of the antigen under reduced conditions. The negative control didn't stain any band in the lysate.

Magnetic cell sorting by the MAbs

The cells were magnetically sorted with Mm or MGm. Most (>95%) of the cells selected by the two MAbs were macrophages and neutrophils, but these cell populations were also observed in the negative fractions (Table 1). After the magnetic separation, neutrophils and macrophages accounted for 99.0% of the Mm-posi-

tive cells (84.8% and 14.2%, respectively), and 95.3% of the MGm-positive cells (77.3% and 18.0%, respectively). On the other hand, the negative cell fractions selected by the two MAbs each were constituted of various cell types, including macrophages and neutrophils. In the MAb-negative fractions, macrophages and neutrophils accounted for 57.6% of the Mm-negative cells (34.3% and 23.3%, respectively), and 38.6% of the MGm-negative cells (34.9% and 3.7%, respectively). The MAb MGm deleted more neutrophils by its binding than did Mm.

Figs. 3-5. Transmission electron micrographs of a neutrophil or a macrophage immunoreacted with the MAbs. **3.** A neutrophil immunoreacted with the MAb Mm. This cell has a segmented nucleus with condensed chromatin. Gold particles (arrows) are visible near the cell membrane and in the cytoplasm. Bar = 2 μ m. **4.** A macrophage immunoreacted with the MAb MGm. This cell has a nucleus with low condensed chromatin and many vesicles in the cytoplasm. Gold particles (arrows) are present on the cell membrane and in the cytoplasm. Bar = 2 μ m. **5.** A neutrophil immunoreacted with the MAb MGm. Gold particles are present near the membrane and in the cytoplasm. Bar = 2 μ m.

Figs. 6–7. 6. Cytoplasm of a macrophage bearing gold particles (arrows) immunoreacted with the MAb MGcp1, showing a nucleus with low condensed chromatin (N), a number of vesicles (v) and microfilaments (f). Bar = 1 μ m. 7. Cytoplasm of a neutrophil bearing gold particles (arrows) immunoreacted with the MAb MGcp2. Bar = 1 μ m.

Fig. 8. SDS-PAGE and immunoblotting of a PEC lysate with the four MAbs. Lane 1: Molecular weight markers. Lane 2: SDS-PAGE profile of PEC lysate. Lane 3: Mm. Lane 4: MGm. Lane 5: MGcp1. Lane 6: MGcp2. Arrowheads indicate immunoreactive bands. Numbers on the left correspond to the molecular weight markers in kDa.

Discussion

In this study, four MAbs (Mm, MGm, MGcp1 and MGcp2) were produced against PEC of rainbow trout and found to react with antigens shared with both macrophages and neutrophils. To date, Slierendrecht *et al.* (1995) and Bowden *et al.* (1997) reported a MAb that cross-reacted with trout granulocytes and thrombocytes and two MAbs that cross-reacted with trout macrophages, granulocytes and lymphocytes, respectively. But no MAb that was specific for only trout macrophages and neutrophils, i.e., phagocytic cells, has been shown. Hence it is considered that the present study is the first report of anti-macrophages and neutrophils MAbs in rainbow trout which should be helpful for isolation and identification of these cell populations more easily.

We used PEC as an immunogen to produce MAbs reactive with macrophages and neutrophils since the PEC was rich in these cell populations (Afonso *et al.*, 1998). Through analyses of IFA, FCM and immuno-

Table 1. Mean percentage $\pm$ S.D. of the cells separated by magnetic cell sorting with Mm or MGm from rainbow trout peritoneal exudate cells (PEC)

MAB	M ϕ * ¹	NP* ²	Lymph.* ³	ER* ⁴	others
Mm: positive fraction	14.2 $\pm$ 2.0	84.8 $\pm$ 2.3	0.6 $\pm$ 0.4	0.1 $\pm$ 0.2	0.3 $\pm$ 0.1
negative fraction	23.3 $\pm$ 1.4	34.3 $\pm$ 1.3	20.8 $\pm$ 3.7	19.8 $\pm$ 5.1	1.8 $\pm$ 0.4
MGm: positive fraction	18.0 $\pm$ 1.5	77.3 $\pm$ 2.1	2.7 $\pm$ 0.8	0.9 $\pm$ 0.5	1.1 $\pm$ 0.4
negative fraction	34.9 $\pm$ 3.8	3.7 $\pm$ 0.9	7.6 $\pm$ 3.2	53.2 $\pm$ 3.0	0.5 $\pm$ 0.3
intact PEC* ⁵	24.9 $\pm$ 2.0	56.3 $\pm$ 2.6	11.0 $\pm$ 1.8	6.6 $\pm$ 2.9	1.2 $\pm$ 0.6

*¹ Macrophages*² Neutrophils*³ Lymphocytes*⁴ Erythrocytes*⁵ Peritoneal exudate cells before magnetic sorting

electron microscopy, it was found that the four MABs reacted with macrophages and neutrophils but not with thrombocytes, lymphocytes and erythrocytes. Identification of the MAB-positive cells under the electron microscope was based on previously described criteria (Ellis, 1977; Suzuki, 1986; Rowley *et al.*, 1988; Ainsworth, 1992; Suzuki and Iida, 1992; Afonso *et al.*, 1997 and 1998). The cells labelled with gold particles were identified as macrophages and neutrophils from their morphological characteristics. Lymphocytes and thrombocytes were thought to be MAB-negative cells because of the absence of gold particles or the absence of specific immunofluorescence.

Further analyses showed the localization of the antigens that can be detected with MGcp1 and MGcp2. The MABs MGcp1 and MGcp2 did not react with living leukocytes in FCM while Mm and MGm clearly showed the immunoreaction with them, indicating the antigens recognized by MGcp1 and MGcp2 were present inside the cells. This fact was also confirmed by immunoelectron microscopy. Gold particles conjugated with MGcp1 or MGcp2 were observed in the cytoplasm, while the complexes of Mm-gold particles and MGm-gold particles were found in the cytoplasm and on the cell surface. These data suggest that MGcp1 and MGcp2 are reactive with intracytoplasmic antigens restricted to macrophages and neutrophils. The antigens recognized by MGcp1 and MGcp2 might exist on molecules related to functions performed in the cytoplasm of macrophages and neutrophils as seen the antigen CD 68 is a lysosomal membrane-associated antigen restricted to macrophages (Parwaresch *et al.*, 1986).

Interestingly, the four MABs did not react with all of the macrophages and/or neutrophils. In the present flow cytometry studies, the trout leukocytes in the FS/SS dot plot patterns were classified according to previous descriptions (Morgan *et al.*, 1993; Kfoury Jr. *et al.*, 1999). The analysis indicated that Mm and MGm reacted strongly with the monocyte/macrophage and neutrophil fraction (region B) but not with all of the cells. The percentages of MAB-positive cells in region B also varied among PEC, HKL and PBL. In the elec-

tron-microscopic observations of cells with each MAB, there were always some macrophages and neutrophils with no gold particles (figures not shown). In addition, in the magnetic sorting experiments, the Mm- and MGm-negative cell fractions included macrophage and neutrophil populations. Macrophages and neutrophils have different morphologies and functions within the same cell population depending on their stage of maturation or state of activation (Suzuki, 1986; Ainsworth, 1992; Secombes and Fletcher, 1992; Matsuyama and Iida, 1999). The MABs produced in this study may have reacted with determinants related to different cell stages in monocytes/macrophages and neutrophils. Further studies of the differences in functions of the MAB-positive and the MAB-negative cells in the macrophage and neutrophil populations are needed, which may clarify characteristics of the cell stages of these cell populations.

In the magnetic sorting with Mm or MGm, more than 95% of the cells in the MAB-positive fractions were macrophages and neutrophils. Separation of these cell populations by using MABs can avoid the disadvantages of separation by cell adherence selection and Percoll centrifugation, meaning unexpected activation of cells due to adherence and contamination of other cell populations such as weakly adherent cells and cells with similar density. Present high efficiency of separation by the MAB can be useful in studying the function of these cells. Magnetic cell sorting with MGm was especially effective in removing neutrophils from the negative fraction. These MABs may be useful in combination with other MABs. For example, separation from intact leukocytes suspension using MGm, which strongly reacts with neutrophils, in combination with other MABs against lymphocytes and/or thrombocytes will be applicable for purification of particular monocyte populations in the negative fraction after the separation.

Regrettably, the ratios of MAB-positive and MAB-negative cells within macrophage and neutrophil population each were not focused in this study. But it was considered that these ratios could be varied among the leukocytes collected from different tissue origins or under stimulated conditions since these four MABs might

react with antigens related to a cell stage within one cell population as described above.

The Western blot analysis under reduced conditions showed that the four MAbs had different reactivities. Mm and MGm showed a number of immunoreactive bands with different molecular weights. These results suggest that the same antigenic determinants are shared by several proteins, as previously reported by Greenlee and Ristow (1993). MGcp1 may recognize three-dimensional structures since the antigen was denatured under reduced conditions while MGcp2 showed one band. It can be concluded that the four MAbs recognize epitopes with different characteristics.

In summary, four MAbs that reacted with antigens shared by rainbow trout macrophages and neutrophils were established, and these cells were successfully isolated from other cells by two MAbs each. These MAbs will be useful tools for future studies of trout immunity involving phagocytes, such as analyses of antigen presentation and cytokine releases. Further studies to analyse the functional differences between MAb-positive and -negative cells would also disclose new knowledge of these cell populations.

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