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ORIGINAL ARTICLES

R-MC46 monoclonal antibody stimulates adhesion and phagocytosis by rat macrophages

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Background. *In our previous experiments it was shown that R-MC46 monoclonal antibody (mAb), produced at our Institute, stimulated homotypic aggregation of rat granulocytes and production of proinflammatory cytokines. The aim of this study was to examine antigen expression and function, recognized by R-MC46 mAb on macrophages.* ***Methods.*** *The expression of R-MC46 antigen on thymic and peritoneal macrophages was investigated using immunocytochemistry and flow cytometry methods. Its biochemical characterization was performed by Western blot. The ability of R-MC46 mAb to modulate adhesion and phagocytosis by macrophages was studied by using co-culture experiments with autologous thymocytes.* ***Results.*** *R-MC46 mAb stained thymic macrophages more strongly than peritoneal macrophages. After in vivo treatment of peritoneal macrophages with Pristane, a significant up-regulation of the R-MC46 antigen expression was observed. Western blot analysis showed that the mAb recognized a low molecular weight antigen of about 5.5 kDa. R-MC46 mAb significantly enhanced binding and phagocytosis of thymocytes by both thymic and peritoneal macrophages. These processes were completely blocked by WT.3 (anti-CD18) mAb. The stimulation of binding thymocyte to macrophages was higher with the use of thymic macrophages,while the phagocytosis of these cells was higher in the presence of peritoneal macrophages.* ***Conclusion.*** *R-MC46 mAb recognized a new molecule expressed by rat macrophages. The antigen is most probably involved in β2 integrin-mediated adhesion and phagocytosis, as well as proinflammatory functions of macrophages.*

Key words: **phagocytosis; adhesiveness; macrophages; antibodies, monoclonal; antigens, CD18.**

Introduction

The mononuclear phagocyte system (monocytes and macrophages) and polymorphonuclear leukocytes are the major cellular effectors of inflammation and tissue injury (1). These processes are mediated by different membrane and intracytoplasmic molecules. Some of them are common in different cells, such as mononuclear phagocytes, endothelial, and epithelial cells (2–4). Many of these molecules are actively involved in the regulation of cell adhesion. Subsequently, adhesive interactions regulate different cell responses, such as transmigration into inflammatory site, cytokine secretion, production, reactive oxygen intermediates, degranulation, and phagocytosis (1).

Macrophages are highly phagocytic cells, which readily engulf and digest a variety of cells, antigens, and pathogens (5, 6). They play important roles in inflammation, responding to a wide range of activation signals and chemotactic factors (7). Macrophages in different tissue and body cavities are heterogeneous in terms of morphology, enzyme activity, cell surface properties, differentiation, and activation states (8, 9). It is believed that the microenvironment of a tissue regulates many of these characteristics of macrophages.

A major step in the field of macrophage research was achieved with monoclonal antibody (mAb) technology (4). MAb have become an invaluable tool for the analysis of macrophage heterogeneity and its activation, as well as for the identification of new molecules expressed by these cells

(2, 10). Our original results on the expression and function of a small molecule on rat peritoneal and thymic macrophages, defined by R-MC46 mAb are presented in this paper. The mAb with unique specificity had been previously produced at our Institute by fusing mouse myeloma cells and splenocytes from mice immunized with rat thymic stroma (3, 11).

Methods

Animals. AO-rats of both sexes 8–10 weeks of age, bred at the Farm for Experimental Animals, Military Medical Academy , Belgrade, were used in this study.

R-MC46 mAb. R-MC46 mAb had been previously produced at our Institute (10). BALB/c mice were immunized intraperitonealy (i.p.) with rat thymic homogenate which contained enriched nonlymphoid cells prepared after *in vivo* injection of hydrocortisone. Splenocytes from immunized mice were fused with P3XAg8 myeloma cells using polyethylene glycol 1500 (PEG, Serva). Hybridomas, produced from the fusion, were selected in hypoxantine-aminopterine-thymidine (HAT) medium (Sigma), and screened by immunoperoxidase staining on cryostat thymic sections. R-MC46 mAb that had been selected according to its characteristic staining pattern was purified from ascites by salt fractionation (1).

Preparation of thymic macrophages. Suspension of thymocytes was obtained by teasing thymuses against a steel mesh in RPMI-1640 medium with 2% fetal calf serum (FCS). After washing, cells were counted and resuspended in complete medium (RPMI 1640 with 10% FCS). Cells were placed on multi-spot glass slides (Flow) (2–2,5 × 10^5cells/spot), and cultivated in an incubator with 5% CO_2 at 37 °C. After two days, non-adherent cells were discarded and adherent cells – predominantly macrophages, as checked by ED1 mAb staining (12), were used for immunocytochemistry, rosette and phagocytosis assay.

Preparation of peritoneal macrophages. Peritoneal cells were harvested from rat peritoneal cavity lavaged with 20ml RPMI 1640 medium (ICN Flow, USA). Stimulation of peritoneal cells was induced by i.p. injection of 1ml Pristane (2, 6, 10, 14 - tetramethyl pentadecane, Serva) in rats. Harvesting was performed 4 days later as described. Macrophages were enriched from peritoneal cells using the Optiprep gradijent™ (Nycomed Pharma AS, Oslo, Norwey). Cells prepared in such a way were plated on multi-spot glass slides (2–2,5 × 10^4cells/spot) at 37 °C, 5% CO_2. After incubating cells for 2 hrs, non-adherent cells were discarded, and adherent cells were used for further experiments.

Preparation of thymocytes. For binding and phagocytosis assays, thymocytes were prepared by teasing thymuses against a steel mesh. Released cells were collected and resuspended in RPMI 1640 medium containing 10% FCS. The suspension was filtered through a nylon gauze to remove fibrous residues, washed, and counted. Cell viability (higher than 95%) was determined by trypan blue dye exclusion.

Adhesion and phagocytosis assay. Multispot glass slides with adherent thymic and peritoneal macrophages were cultivated for 30 min with R-MC46 mAb (5 μg/ml), in control medium, or with an irrelevant cBH9 mAb. For blocking experiments, macrophages were preincubated with WT3 (an anti-CD18) mAb, a gift from Dr Miyasaka M, Osaka, Japan. In the next step, thymocytes (5 × 10^5 cells/spot) were added to macrophages and cultivated at 37 °C. After 30 min of incubation (rosette assay), thymocytes were removed by gentle washing with phosphate-buffered saline (PBS). After 4hrs of incubation (phagocytosis assay) slides were vigorously washed with PBS to remove unbound and bound thymocytes. After that, slides were fixed in 4% formaldehyde and stained with hematoxylin/eosin. Macrophages that bounded three or more thymocytes were scored as rosettes. For each assay, 500 macrophages were counted. The percentage of rosettes was determined on the basis of total macrophages and macrophages forming rosettes. The rosette index was calculated by dividing the total number of bound thymocytes to macrophages with total number of macrophages. Macrophages that phagocytosed three and more thymocytes were scored as phagocytic macrophages. Phagocytosis was also measured as phagocytosis index by dividing the total number of thymocytes ingested by macrophages with the total number of macrophages.

Flow cytometry. For immunofluorescence staining peritoneal cells (5 × 10^5 cells) were incubated with R-MC46 mAb (5 μg/ml) at 4 °C for 45 min. After incubation and subsequent washing with PBS containing 2% FCS and 0.1% sodium azide, the cells were stained with fluoresceine isothiocyanate (FITC)-conjugated anti-mouse Ig (Serotec, UK) diluted in 5% rat serum at 4 °C, for 30 min. Control cells were incubated with the secondary antibody alone. Finally, cells were fixed with 1% formaldehyde and analyzed on an EPICS XL-MCL flow cytometer (Coulter, Germany). Macrophages were identified by characteristic side scatter versus forward scatter profile (11).

Immunocytochemistry. Adherent macrophages were fixed with acetone and incubated with R-MC46 mAb (5 μg/ml) for 60 min. Then, the slides were washed twice with Tris buffered saline (TBS) and subsequently were incubated for 30 min with secondary antibody (anti-rabbit Ig, DAKO A/S Denmark) diluted in 5% rat serum. Afterwards, the slides were washed and treated using alkaline phosphatase - antialkaline phosphatase complex method (APAAP, DAKO A/S Denmark). The reaction was visualized using the substrate of alkaline phosphatase (Fast Red Substrate System, DAKO A/S Denmark). Finally, the slides were lightly counterstained with hematoxylin.

Western-blot. Peritoneal macrophages (30 × 10^6) were washed twice with PBS. The pellet was lysed in 300 μl of buffer containing 1% detergent Nonidet P-40 (NP-40, Sigma, Germany) and a coctail of protease inhibitors (Sigma, Germany) that were held on ice for 15 min. Extracts were separated on 15% polyacrylamide gel and transfered onto a PVDF

membrane (Sequi-blot ™PVDF membrane 0.2 μm) using the Bio-Rad transblot system. PVDF sheets were blocked for 1hrs in 3% gelatine-TBS. Blots were probed with R-MC46 mAb and then with secondary antibody (rabbit anti-mouse Ig), conjugated with peroxidase (Santa Cruz Biotechnology). The peroxidase reaction was visualized using 0.06% diaminobenzidine (DAB, Serva) and 0.01% H_2O_2.

Results

R-MC46 mAbs recognizes a 5.5 kDa antigen expressed by rat macrophages

Thymic and peritoneal macrophages were stained with R-MC46 mAb. Fig. 1 shows that almost all thymic macrophages were R-MC46$^+$. In contrast, only a half of peritoneal macrophages were stained with the mAb. The expression of R-MC46 antigen by thymic macrophages was stronger than its expression by peritoneal macrophages.

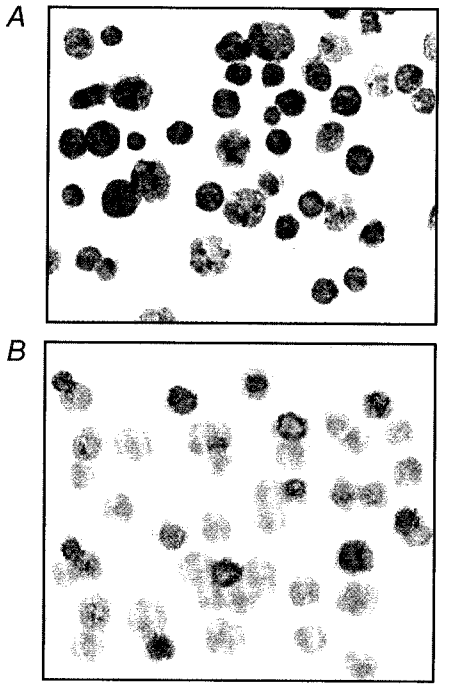

Fig. 1 – Expression of the R-MC46 antigen by thymic (A) and peritoneal (B) macrophages.

Monolayers of macrophages were stained with R-MC46 mAb using an APAAP method. Note stronger staining of thymic macrophages. Magnification × 400.

Flow cytometric analysis showed that 30–50% of resting peritoneal macrophages was stained with R-MC46 mAb. The *in vivo* treatment of rats with Pristane resulted in up-regulation of the R-MC46 antigen expression on the cell surface of these cells. As seen in Fig. 2, more than 90% of pristane-treated peritoneal macrophages represented R-MC46$^+$.

Western blot of lysate from peritoneal macrophages showed that R-MC46 mAb recognized a single polypeptide of 5.5 kDa molecular weight (Fig. 3).

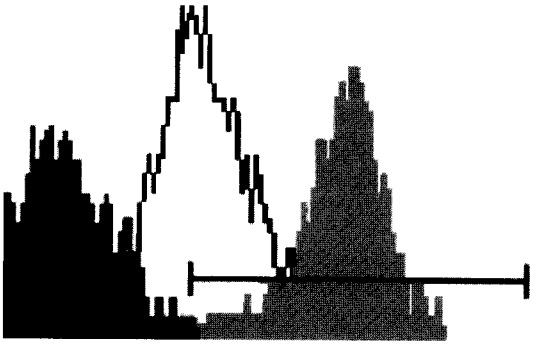

Fig. 2 – Fluorescence profile of the R-MC46 antigen expression on rat peritoneal macrophages.

Cells were stained in suspension with R-MC46 mAb and then analyzed by flow cytometry. Black histogram represents the level of non-specific staining (isotypic control). White and gray histograms show the fluorescence profile of non-activated and pristane-activated macrophages, respectively. Horizontal bar determines the levels of specific fluorescence. Note up-regulation of the R-MC46 expression by pristane-stimulated macrophages.

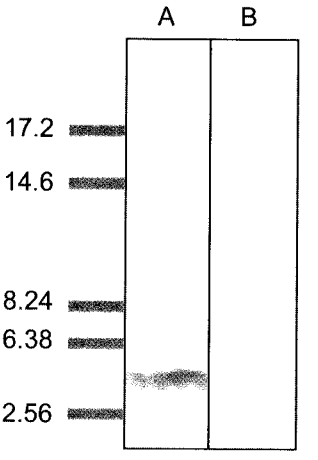

Fig. 3 – Western blot of lysate from peritoneal cells stained by R-MC46 mAb.

Lysates were resolved on 15% SDS-PAGE under reducing conditions, transferred to PVDF membrane and stained with R-MC mAb (A), and control secondary antibody (B). Note that R-MC46 mAb recognizes a single polypeptide aprox. 5.5 kDa mol. weight.

R-MC46 mAb stimulates binding of thymocytes to and phagocytosis by macrophages

In the next experiments we tested the ability of R-MC46 mAb to modulate adhesion of thymocytes to macrophages. Results presented in Fig. 4 and Fig. 5 shows that the antibody stimulated binding of thymocytes to both peritoneal and thymic macrophages. An irrelevant mAb (cBH9) did not influence the binding process. Thymic macrophages had higher binding capacity for thymocytes than peritoneal macrophages.

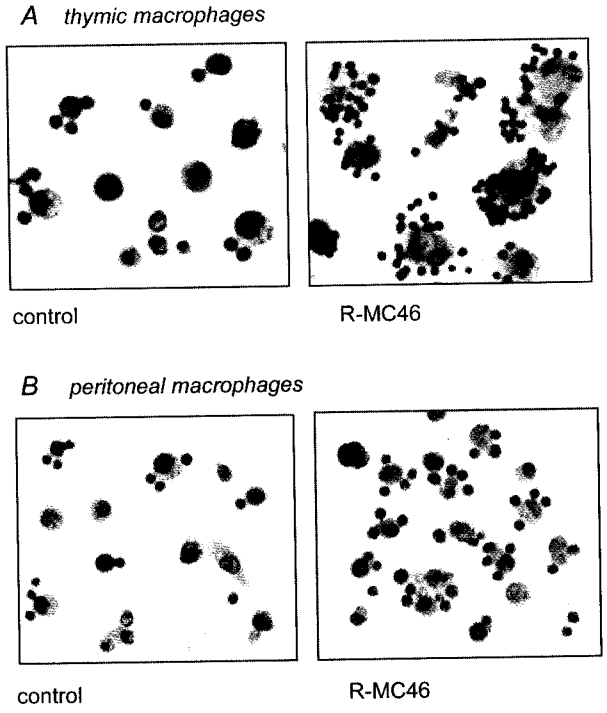

Fig. 4 – Binding of thymocytes to thymic (A) and peritoneal macrophages (B).

Cellular adhesion was performed using a rosette assay in the presence of R-MC46 mAb or medium alone. Macrophages that bound three or more thymocytes were scored as rosettes. Note significantly higher binding of thymocytes to macrophages in the presence of R-MC46 mAb. Magnification: × 400.

After prolonged incubation certain thymocytes were phagocytosed by macrophages. As expected, R-MC46 mAb significantly stimulated phagocytosis of thymocytes. However, in contrast to previous findings, peritoneal macrophages activated with R-MC46 mAb had higher phagocytic capacity (Fig. 6 and Fig. 7).

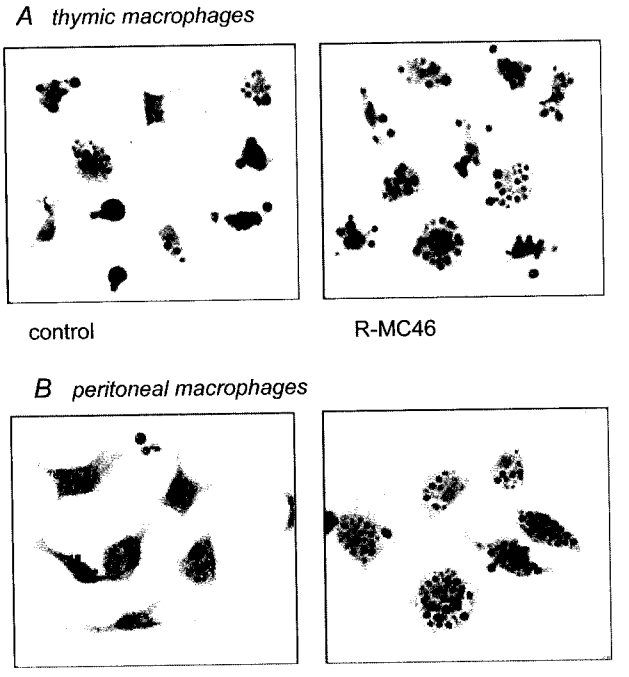

Fig. 6 – Phagocytosis of thymocytes by thymic (A) and peritoneal macrophages (B).

Phagocytosis was done using a phagocytosis assay in the presence of R-MC46 mAb or medium alone. Note significantly higher phagocytosos of thymocytes by macrophages in the presence of R-MC46 mAb. Magnification: × 400.

Effect of anti-CD18 mAb on adhesion and phagocytosis processes by macrophages

Finally, we investigated whether adhesion and phagocytosis processes stimulated with R-MC46 mAb, as shown in previous experiments, were dependent on the engagement of CD18. As presented in Fig. 8, WT3 (anti-CD18 mAb) completely blocked thymocyte binding and phagocytosis by both thymic and peritoneal macrophages.

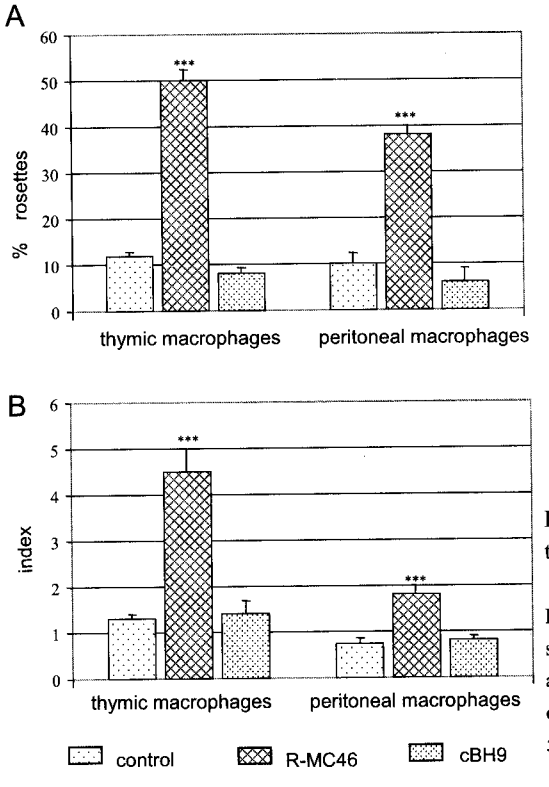

Fig. 5 – Effect of R-MC46 mAb on binding of thymocytes in culture to thymic and peritoneal macrophages.

Binding was assesed using the rosette assay after the cultivation of cells. Histogram A represents the percentage of rosettes, whereas histogram B represents the adhesion index (number of thymocytes bound per macrophage). Values are given as mean ± SD of quadruplicates from one representative experiment, out of several ones with similar results. Results are given on the basis of 500–700 calculated cells on each cytospin preparation.

(*** $p < 0.001$ compared to medium control or irrelevant mAb, cBH9).

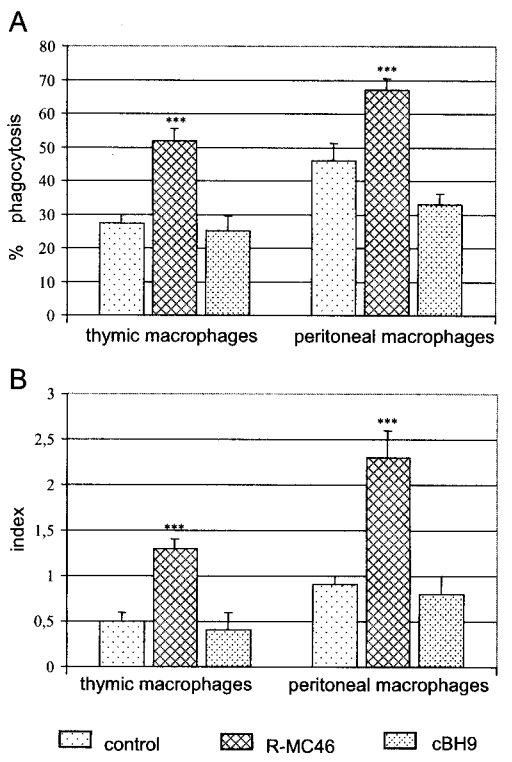

Fig. 7 – Effect of R-MC46 mAb on phagocytosis of thymocytes in culture to thymic and peritoneal macrophages.

Phagocytosis was assesed using phagocytosis assay after cultivation of cells. Histogram A represents the percentage of phagocytosis whereas histogram B represents the index of phagocytosis (number of thymocytes phagocytosed by one macrophage). Values are given as mean ± SD of quadruplicates from one representative experiment, out of five ones with similar results. Results are given on the basis of 500–700 calculated cells on each cytospin preparation.

(*** p< 0.001 compared to medium control or irrelevant mAb, cBH9).

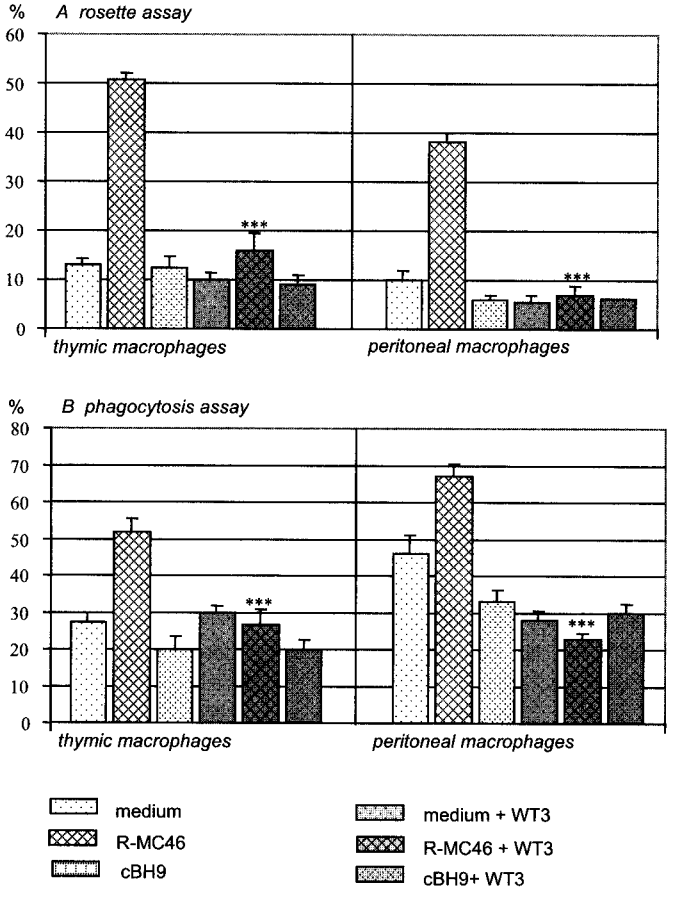

Fig. 8 – Effect of WT.3 (anti-CD18) mAb on rat macrophage adhesiveness (A) and phagocytosis (B).

Macrophages, were preincubated with WT.3 mAb for 30 min before the addition of thymocytes. Adhesion and phagocytosis was measured as already described. Values are given as mean±SD of quadruplicates from one typical experiment, out of three ones with similar results. Results are given on the basis of 500–700 calculated cells on each cytospin preparation. (*** p< 0.001 comparated to R-MC46 mAb).

Discussion

In our previous papers we presented the initial characterization of R-MC46 mAb (3, 11, 13), as well as the mAb, which was raised against rat thymic stroma stains predominantly granulocytes, about 50% of monocytes, different populations of macrophages, thymic epithelium and high endothelial venules in lymph nodes (11). Biochemical analysis showed that R-MC46 mAb recognized a small 3.5–5.5 kDa polypeptide on granulocytes.

Our efforts to identify the nature of the molecule recognized by R-MC46 mAb were unsuccessful. The survey of literature data regarding tissue distribution, physical and chemical characteristics (2–4, 11), indicated that R-MC46 mAb detected an unknown molecule with unique properties.

The experiments presented in this work were been initiated by our previous results showing that R-MC46 mAb induced strong homotypic aggregation of granulocytes, followed by their activation and production of proinflammatory cytokines (12). Supernatants from granulocytes treated *in vivo* with R-MC46 mAb enhanced wound-healing processes (14). These results suggested that the R-MC46 antigen was relevant for different proinflammatory functions of granulocytes.

In this work we showed that the molecule recognized by R-MC46 mAb on macrophages, as determined by Western blot, was the same as the molecule on granulocytes. In addition, up-regulation of its expression by proinflammatory stimuli induced by intraperitoneal application of pristane, suggested that R-MC46 antigen might play a proinflammatory role of macrophages.

A significant part of this investigation was addressed to the role of R-MC46 mAb in mediating adhesive interactions of macrophages. By using a model of heterotypic adhesion between macrophages and thymocytes, we found that R-MC46 mAb significantly stimulated adhesion be-

tween these two cell types. The co-culture experiments between macrophages and thymocytes are a useful model for studying molecules involved in binding and subsequent phagocytosis processes (15, 16). Namely, thymocytes undergo rapid apoptosis *in vitro*. Their removal by macrophages represents an *in vitro* equivalent of the *in vivo* process in the thymus. *In vivo* thymic macrophages remove thymocytes in which apoptotic processes are triggered by negative selection or due to unsuccessful positive selection (17). The initial binding of thymocytes to macrophages is followed by their phagocytosis. In this work, we showed that R-MC46 mAb significantly enhanced phagocytosis of thymocytes not only by thymic but also by peritoneal macrophages. These results suggested that the behavior of both types of macrophages was based on the same non-specific recognition/engulfment mechanisms, related to the well-known role of macrophages in the innate immunity (18, 4, 19).

Our results demonstrated that peritoneal macrophages had lower binding properties, but higher phagocytic potential for thymocytes than thymic macrophages. These differences could be explained by different activation state of macrophages. Thymic macrophages that were isolated after two days of culture with autologous thymocytes had higher expression of R-MC46 antigen than freshly isolated peritoneal macrophages, probably due to previous activation of these cells in culture. It is also possible that macrophages, within specific thymic microenvironment, express additional adhesion molecules that allow them better and faster removal of non-functional thymocytes (20, 21). One of these molecules that modulate adhesion processes could be R-MC46. The level of R-MC46 antigen expression directly correlated with adhesion capability of macrophages. In contrast, lower basal and R-MC46 mAb-induced phagocytic capacity of thymic macrophages, compared to peritoneal macrophages, could be explained by the fact that phagocytosis of thymocytes by thymic macrophages had been previously triggered in co-culture with autologous thymocytes during the preparation of macrophage monolayers. Their repeated exposure to dying thymocytes resulted in the reduction of phagocytosis processes.

What are possible mechanisms by which R-MC46 mAb increases adhesive interactions of macrophages? R-MC46 antigen is too small to be an adhesion molecule, because neither of the identified adhesion receptors possesses such characteristics (11, 22, 23). The hypothesis that R-MC46 mAb-induced adhesive interactions are completely blocked by an anti-CD18 mAb (WT3) (13) is also based on our present heterotypic adhesion experiments and previous findings in a model of homotypic aggregation of granulocytes. CD18 is the common β-chain of β2 integrins. Macrophages express at least three molecules of the β2 integrin family: CD11a/CD18 (LFA-1), CD11b/CD18 (Mac 1) and variable/weakly CD11c/CD18 (1). Among these integrins, the expression of CD11b/CD18 is most prominent. In contrast, thymocytes express only CD11a/CD18 (23). It can be postulated that the binding of R-MC46 mAb to the respective antigen triggers signalling pathways in macrophages that resulted in the activation of β2 integrins. The activation of β2 integrins that is mediated through "inside-out" signalling is an unspecific process because the engagement of a number of cell surface molecules by specific mAbs generates proadhesive signals that activate β2 integrins, especially LFA-1 (24). It has been suggested that these intracellular signals enhance the affinity and avidity of β2 integrins either by conformational changes or by multimerization at the cell surface (25, 26). For CD11b/CD18, up-regulation of its expression on cell membrane is also caused by translocation from intracellular stores (1). At the present, the ligands on thymocytes to which β2 integrins on macrophages bind are not identified. One of the candidates could be intercellular adhesive molecule-1 (ICAM-1). ICAM-1 is present on about 20% resting thymocytes, but its expression is up-regulated upon its activation (23).

In conclusion, our results indicated that R-MC46 antigen, up to now an unknown molecule, was involved in adhesive and phagocytic processes of macrophages. These mechanisms could have different significance in the immune system depending on the microenvironment in which macrophages are located, as well as their specific proinflammatory or immunoregulatory functions.

REFERENCES

1. *Berton G, Lowell CA.* Integrin signalling in neutrophils and macrophages. Cell Signal 1999; 11(9): 621–35.
2. *Knapp W.* Myeloid section report. In: *Knapp W, Dorkien B, Gilks WR, Reiber EP, Schmidt RE, Stein H,* et al, editors. Leucocyte Typing IV. White Cell Differentiation Antigens. Oxford: Oxford University Press; 1989. p. 747–80.
3. *Čolić M, Gašić S, Popović Lj, Dujić A.* Identification of molecules shared between subcapsular/medullary epithelium, granulocytes and a subset of macrophages in rat thymus. In: *Imhof BA, Berrih-Aknin S, Ezine S,* editors. Lymphatic Tissues and In Vivo Immune Responses. New York: Marcel Dekker, Inc.; 1991. p. 61–8.
4. *Hume AD, Ross IL, Himes SR, Sasmono RT, Wells CA, Ravasi T.* Mononuclear phagocyte system revisited. J Leukoc Biol 2002; 72(4): 621–7.
5. *Johnson RB Jr.* Current concepts: immunology. Monocytes and macrophages. N Engl J Med 1988; 318(12): : 747–52.
6. *Laskin DL, Weinberger B, Laskin JD.* Functional heterogeneity in liver and lung macrophages. J Leukoc Biol 2001;70(2): 163–70.

7. *Oghiso Y, Yamada Y, Shibata Y.* Exudation of proliferative macrophages in local inflammation in the peritoneum. J Leukoc Biol 1992; 52(4): 421–4.

8. *Auger MJ, Ross JA.* The biology of the macrophage. In: *Lewis CE, McGee JO'D,* on editors. The Macrophage. Oxford: Oxford University Press; 1992. p. 27–30.

9. *Gordon S, Clarke S, Greaves D, Doyle A.* Molecular immunobiology of macrophages: recent progress. Curr Opin Immunol 1995; 7(1): 24–33.

10. *Čolić M, Popović Lj, Gašić S, Dragojević-Simić V, Milićević NM, Matanović D,* et al. Immunohistochemical characterization of rat thymic non-lymphoid cells. II. Macrophages and granulocytes defined by monoclonal antibodies. Immunology 1990; 69(3): : 416–22.

11. *Gašić S.* Production and characterization of R-MC46 and R-MC47 monoclonal antibodies reactive to non-lymphoid cells of rat lymphatic organs [master's thesis]. Belgrade: Faculty of Mathematics and Natural Science; 1994. (in Serbian)

12. *Dijkstra CD, Dopp EA, Joling P, Kraal G.* The heterogenity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. Immunology 1985; 54(3): 589–99.

13. *Pejnović N, Čolić M, Drašković-Pavlović B, Dujić A.* A monoclonal antibody R-MC46 induces homotypic adhesion and activation of rat peripheral blood neutrophils. In: *Lukić ML, Čolić M, Mostarica-Stojković M, Čuperlović K,* editors. Immunoregulation in Health and Disease. London: Academic Press; 1997. p. 95–101.

14. *Pejnović N.* Modulation of wound healing process by soluble products of granulocytes activated by monoclonal antibodies under conditions of severe injury [dissertation]. Belgrade: Military Medical Academy; 1993. (in Serbian)

15. *Kyewski B.* Unravelling the complexity of intrathymic cell-cell interactions. APMIS 1988; 96(12): 1049–60.

16. *Čolić M, Drabek D.* Expression and function of intercellular adhesion molecule 1 (ICAM-1) on rat thymic macrophage in culture. Immunol Lett 1991; 28(3): : 251–7.

17. *Henson PM, Bratton DL, Fadok VA.* Apoptotic cell removal. Curr Biol 2001; 11(19): R795–805.

18. *Aderm A, Underhill DM.* Mechanisms of phagocytosis in macrophages. Annu Rev Immunol 1999; 17: : 593–623.

19. *Mosser DM.* The many faces of macrophage activation. J Leukoc Biol 2003; 73(2): 209–12.

20. *Moffatt OD, Devitt A, Bell ED, Simmons DL, Gregory CD.* Macrophage recognition of ICAM-3 on apoptotic leukocytes. J Immunol 1999; 162(11): 6800–10.

21. *Callahan MK, Williamson P, Schlegel RA.* Surface expression of phosphatidylserine on macrophages is required for phagocytosis of apoptotic thymocytes. Cell Death Differ 2000; 7(7): 645–53.

22. *Harris ES, McIntyre TM, Prescott SM, Zimmerman GA.* The leukocyte integrins. J Biol Chem 2000; 275(31): 23409–12.

23. *Bluestone JA, Khattri R, van Seventer GA.* Accessory molecules. In: *Paul WE,* editor. Fundamental Immunology. 4th ed. Philadelphia: Lippincott-Raven; 1999. p. 449–78.

24. *Čolić M, Ilić V, Tamatani T, Miyasaka M, Pavlović MD.* Role of beta 2 integrins in the binding of thymocytes to rat thymic macrophages. Dev Immunol 1994; 4(1): 65–77.

25. *Simon SI, Cherapanov V, Nadra I, Waddell TK, Seo SM, Wang Q,* et al. Signaling functions of L-selectin in neutrophils: alterations in the cytoskeleton and colocalization with CD18. J Immunol 1999; 163(5): : 2891–901.

26. *Hogg N, Leitinger B.* Shape and shift changes related to the function of leukocyte integrins LFA-1 and Mac-1. J Leukoc Biol 2001; 69(6): 893–8.

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Apstrakt

Gašić S, Vučević D, Popović P, Vasilijić S, Čolić M. Vojnosanit Pregl 2004; 61(6): : 581–588.

MONOKLONSKO ANTITELO R-MC46 STIMULIŠE ADHEZIJU I FAGOCITOZU OD STRANE MAKROFAGA PACOVA

Uvod. U prethodnim eksperimentima pokazano je da monoklonsko antitelo (mAt) R-MC46, stvoreno u našem Institutu, stimuliše homotipsku agregaciju granulocita pacova i stvaranje proinflamatornih citokina. Cilj ovog rada bio je da se ispitaju ekspresija i funkcija antigena kojega prepoznaje R-MC46 mAt na makrofagama. **Metode.** Ekspresija antigena R-MC46 je praćena pomoću imunocitohemijskih i floucitometrijskih metoda. Biohemijska karakterizacija antigena je izvršena Western-blot analizom. Sposobnost R-MC46 mAb da moduliše adheziju i fagocitozu ispitivana je

pomoću eksperimenata kokultivacije makrofaga sa autolognim timocitima. **Rezultati.** Pokazano je da se R-MC46 mAt jače vezuje za timusne nego za peritoneumske makrofage. Nakon *in vivo* tretmana Pristanom kod peritoneumskih makrofaga je uočena značajno veća ekspresija R-MC46 antigena. Western-blot analizom je pokazano da mAt prepoznaje antigen male molekulske mase, od oko 5,5 kDa. R-MC46 mAt je značajno stimulisalo vezivanje i fagocitozu timocita od strane kako timusnih tako i peritoneumskih makrofaga. Ovi procesi bili su kompletno blokirani sa WT.3 (anti-CD18) mAt. Stimulacija vezivanja timocita za makrofage bila je izraženija korišćenjem timusnih makrofaga, dok je fagocitoza ovih ćelija bila veća u prisustvu peritoneumskih makrofaga. **Zaključak.** R-MC46 mAt prepoznaje novi molekul koji je ispoljen na makrofagama pacova. On je verovatno uključen u procese adhezije i fagocitoze koji su posredovani β2 integrinima, kao i u proinflamatorne funkcije makrofaga.

K l j u č n e r e č i : **fagocitoza; adhezivnost; makrofagi; antitela, monoklonska; antigeni, CD18.**

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