A Monoclonal Antibody against Chicken Monocytic Leukemia Cell Line

Haruo MATSUDA, Makoto INOUE¹⁾ Munetaka NEGORO, and Masayoshi MURATA

Department of Microbiology and Hygiene, Faculty of Applied Biological Science, Hiroshima University, Shitami, Saiji-cho, Higashi-Hiroshima 724 and ¹⁾Department of Veterinary Pathology, Faculty of Agriculture, Yamaguchi University, Yoshida 1677-1, Yamaguchi 753, Japan

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Cells of the monocyte/macrophage lineage have an important role as essential accessory cells for immune system. A chicken non-lymphoid cell line, IN24, which has been established from a spontaneous myelocytic leukemia was identified as a monocytic leukemia cell line from the morphological and functional properties, such as higher adherence on plastic substrates, development of petal-like ruffles on the cell surface, presences of phagocytic and non-specific esterase activities and expression of Fc receptor [7]. Therefore, it seems that a continuous culture of the IN24 cells is useful as homogenous cells of the monocyte/macrophage lineage in chickens.

The present study was designed to produce hybridomas secreting monoclonal antibodies (MoAbs) against antigens associated with the chicken monocyte/macrophage using the IN24 cell line. This report presents the results of development of a MoAb recognized with the IN24 cell line and of flow-cytometric analyses for specificity of the MoAb against various chicken cells.

A 6-week-old female BALB/c mouse was immunized intra-peritoneally with 3×10^6 IN24 cells in phosphate-buffered saline (PBS). One month later, the mouse was boosted intravenously with 5×10^6 IN24 cells, and somatic cell fusion was carried out 3 days later by a modification of basic methods described by Galfre *et al.* [5]. Spleen cells from immunized animal were fused with Sp2/0-Ag 14 myeloma cells. The resulting hybridomas were selected with HAT medium. The details were described previously [10]. Cultures producing antibody positive for IN24 cells were screened by the membrane immunofluorescence test using fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse immunoglobulin (Ig) (Cappel Laboratories, West Chester, PA). In the following screen-

ing, cultures of negative for thymus and bursa cells from normal chickens at 2 weeks of age were selected. After cloning of positive hybridomas in limiting dilution, one hybridoma was selected and referred to as HUM1.

The further specificity of the HUM1 MoAb was determined based on the reactivities against various normal chicken cells and tumor cell lines on flow-cytometry by using an Ortho Cyturon (Ortho Diagnostic Systems, Inc., Tokyo). Thymus, bursa and spleen cells and peripheral blood leukocytes (PBL) as normal cells were prepared from three 4-week-old chickens. Single cell suspensions from thymus, bursa and spleen were prepared as described previously [9]. PBL was obtained from heparinized blood by separation on Ficoll-Paque. A total of 6 cell lines, 3 from Marek's disease virus (MDV)-induced T cell lines, 2 from avian leukosis virus (ALV)-induced B cell lines and the IN24 cell line were used as the chicken tumor cell lines. Two MDV-induced T cell lines, MDCC-MSB1 [1] and MDCC-RP1 [12], were provided by Dr. Nakajima (Osaka Univ., Japan). MDCC-MSB1-41C was isolated from MSB1 by us [11]. Two ALV-induced B cell lines, LSCC-TLT1 [3] and LSCC-1104B1 [6], were supplied by Dr. Mikami (Tokyo Univ., Japan) and Dr. Nii (Okayama Univ., Japan), respectively. The cells from these lines were cultured in RPMI-1640 medium supplement with 10% fetal bovine serum at 38.5°C in an atmosphere containing 5% CO₂. For indirect immunofluorescence, the cells were incubated with undiluted HUM1 supernatant followed by FITC-conjugated anti-mouse Ig. Finally, the cells were subjected for flow cytometry and relative immunofluorescence intensities were measured. Distribution of cells reactive with the HUM1 MoAb in tissues from various lymphoid organs was examined on frozen sections of these organs by using a Vectastain ABC kit (Vector Laboratories, Burlingame, CA) as specified by the manufacture.

The results of reactivities of the HUM1 MoAb

Table 1. Expression of HUM1-positive antigen on normal and tumor cells

Cell ^{a)}	HUM1-positive cells (%)
Normal cells ^{b)}	
Thymus	2.3 ^{c)}
Bursa	<1.0
Spleen	<1.0
PBL	27.8
Tumor cells	
MDCC-	
MSB1	<1.0
MSB1-41C	<1.0
RP1	<1.0
LSCC-	
TLT1	<1.0
1104B1	<1.0
Monocytic cell line	
IN24	65.7

a) Sample cells suspended in phosphate-buffered saline containing 0.02% sodium azide were subjected for flow cytometry. 5,000 to 10,000 cells were calculated per sample.

b) Cells were obtained from three 4-week-old normal chickens.

c) Mean value from three separate experiments.

against various chicken cells are summarized in Table 1. In normal chicken cells examined, bursa and spleen cells contained very low numbers of HUM1-positive cells, whereas the positive cells were detectable in 2.3% of thymus cells and 27.8% of PBL. On the other hand, although the HUM1-positive cells of all lymphoblastoid cell lines (MSB1, MSB1-41C, RP1, TLT1 and 1104B1) examined were each detected in less than 1% of these cells, the IN24 cell line was highly reactive for the HUM1 MoAb. The results indicate that the HUM1 MoAb recognizes antigens expressed commonly on a monocytic leukemia cell line and a certain population of normal cells. On the other hand, in the immuno-histochemical examinations, frozen sections from the thymus, bursa and spleen did not react with the MoAb. The different results obtained in the thymus by two methods (flow-cytometry and immuno-histochemical method) may be due to the difference of sensitivity between them. However, as the present study was not comparing these two immunological analyses in detail, no final conclusion could not be reached on their discrepancy.

To identify the normal cells which was reactive with the HUM1 MoAb, PBL from two 4-week-

old normal chickens were analyzed according to flow-cytometric light scatter. Profiles of bidirectional control maps from these samples were resemble each other, and one of them is shown in Fig. 1A. The profile was able to classify into three different cell types. From flow cytometric analysis of chicken PBL by Eerola *et al.* [4], it is considered that cell types into three bit maps, a, b and c, encircled with thick dot lines in Fig. 1A, are lymphocytes and thrombocytes, monocytes and granulocytes, respectively. Subsequently, reactivity of the HUM1 MoAb with these three type cells was examined. The fluorescence profiles of the three type cells are illustrated in Fig. 1B. In the three type cells (Fig. 1B-a, b, c), the percentages of the HUM1-positive cells were 17.7, 72.3 and 93%, respectively. The results suggest that the HUM1 MoAb may be reactive for monocytes and granulocytes rather than lymphocytes and thrombocytes.

The results presented here indicate that the HUM1 MoAb specifically reacts for monocytes of the monocyte/macrophage lineage. Furthermore, high-reactivity of the HUM1 with cells which are considered granulocytes indicates the close relationship between monocytes and granulocytes. In the cluster of differentiation (CD) of human leukocytes, many MoAbs which are common-reactive for monocytes and granulocytes were classified as CD13, CD15, CD35, CD11a and CD11b, respectively [2, 13]. The fact strongly supports the possible relationship between monocytes and macrophages in the chicken.

Recently, Jeurissen *et al.* [8] have established a MoAb, CVI-CHNL-68.1, which recognizes cells of the chicken monocyte/macrophage lineage. From histochemical and immuno-histochemical examinations, they concluded that the MoAb specifically reacts with monocytes, macrophages and interdigitating cells. However, the MoAb reacted with thymus cells (2.6%), spleen cells (10.7%), peritoneal cells (16.8%) and cells (<1%) from bone marrow and PBL in the membrane immunofluorescence [8]. Therefore, their MoAb might be specific for macrophages rather than peripheral blood monocytes. The different reactivities of the HUM1 and CVI-CHNL-68.1 MoAbs against various normal chicken cells suggest that antigens recognized with these MoAbs are independent each other and it may be possible to differentiate between

Fig. 1. Light scatter profile of chicken PBL (A) and fluorescence intensity profiles of the HUM1 MoAb for PBL (B) divided into three different cell types showing in (A); lymphocytes and thrombocytes (a), monocytes (b) and granulocytes (c). In (B), the solid histograms represent staining with the HUM1 MoAb, the dotted lines indicate the control treated with the second antibody alone and the vertical dotted lines indicate background fluorescence intensity.

monocytes and macrophages by using these MoAbs.

The HUM1 MoAb presented here may be useful for ontogenic study of the chicken monocyte/macrophage lineage and for analyses of pathogenesis of avian diseases. For these useful applications, however, further characterizations of this MoAb will be required.

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要 約

鶏単球性白血病細胞株に対する単クローン抗体(短報): 松田治男・井上 誠¹⁾・根来宗孝・村田昌芳(広島大学生物生産学部, ¹⁾山口大学農学部)——鶏単球性白血病細胞株(IN24)に対するマウス単クローン抗体(HUM 1)を作出し, その特異性をフローサイトメーター(FCM)を用いて調べた. HUM 1 抗体の性状並びに腫瘍細胞に対する反応性を調べたところ, この抗体は胸腺細胞の2.3%, 末梢血細胞(PBL)の27.8%及びIN24細胞株の65.7%と反応した. PBL は, FCM 解析から大きく(a)リンパ球・栓球, (b)単球及び(c)顆粒球に分けられ, HUM 1 抗体は, これらのうち主として(b)及び(c)と反応した.