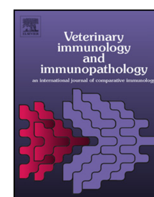
Veterinary Immunology and Immunopathology 178 (2016) 57–63

Contents lists available at ScienceDirect

Veterinary Immunology and Immunopathology

journal homepage: www.elsevier.com/locate/vetimm



Characterization and use of new monoclonal antibodies to CD11c, CD14, and CD163 to analyze the phenotypic complexity of ruminant monocyte subsets

Mahmoud M. Elnaggar[a,b], Gaber S. Abdellrazeq[a,b], Victoria Mack[a], Lindsay M. Fry[a,c], William C. Davis[a], Kun Taek Park[a,d,*]

[a] Department of Veterinary Microbiology and Pathology, College of Veterinary Medicine, Washington State University, WA, USA
[b] Department of Microbiology, Faculty of Veterinary Medicine, Alexandria University, Egypt
[c] Animal Disease Research Unit, U.S. Department of Agriculture, Agricultural Research Service, Pullman, WA, 99164, USA
[d] Department of Veterinary Microbiology, College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 25 February 2016
Received in revised form 7 June 2016
Accepted 27 June 2016

Keywords:
CD11c
CD14
CD16
CD163
Monocytes
Macrophages
Monoclonal antibody

ABSTRACT

The sequencing of the bovine genome and development of mass spectrometry, in conjunction with flow cytometry (FC), have afforded an opportunity to complete the characterization of the specificity of monoclonal antibodies (mAbs), only partially characterized during previous international workshops focused on antibody development for livestock (1991, Leukocyte Antigens in Cattle, Sheep, and Goats; 1993, Leukocyte Antigens of Cattle and Sheep; 1996, Third Workshop on Ruminant Leukocyte Antigens). The objective of this study was to complete the characterization of twelve mAbs incompletely characterized during the workshops that reacted with molecules predominantly expressed on bovine monocytes and use them to provide further information on the phenotypic complexity of monocyte subsets in ruminants. Analysis revealed that the mAbs could be grouped into three clusters that recognize three different molecules: CD11c, CD14, and CD163. Following characterization, comparison of the patterns of expression of CD14 and CD163 with expression of CD16, CD172a, and CD209 revealed the mononuclear cell population is comprised of multiple subsets with differential expression of these molecules. Further analysis revealed the epitopes recognized by mAbs to CD14 and CD163 are conserved on orthologues in sheep and goats. In contrast to CD14 that is also expressed on sheep and goat granulocytes, CD163 is a definitive marker for their monocytes.

© 2016 Elsevier B.V. All rights reserved.

1. Introduction

Various approaches have been used to develop monoclonal antibodies (mAbs) to cell differentiation molecules [CDM, previously referred to as leukocyte differentiation antigens (LDA)] for research in veterinary species. Our initial approach was to hyperimmunize mice with leukocytes from one or more species and then use flow cytometry (FC) to screen primary cultures of hybridomas for mAbs that recognize different CDMs (Davis et al., 1987, 1984). Screening revealed unique patterns of labeling that could be visualized in two-parameter dot plots, using side light scatter vs. fluorescence (SSC vs. FL). MAbs recognizing the same molecule could be clustered based on patterns of labeling. One or more of the cell lines forming a cluster could then be cloned for further analysis. If the cluster contained mAbs of different isotypes, two-color labeling could be used to verify that the mAbs recognized the same molecule. In instances where the mAbs in the cluster were the same isotype, Zenon second step reagents could be used to compare specificity (Davis and Hamilton, 2008). MAbs that recognize different epitopes on the same molecule form a diagonal pattern of labeling, or, if mAbs recognize the same epitope, one mAb may block labeling by the other (Davis et al., 1995; Park et al., 2015b). Participation in international workshops from 1983 to 1998 and in 2004 facilitated final characterization of many mAbs that are currently available for use in veterinary research (Haverson et al., 2001a; Howard et al., 1991; Lunn et al., 1996; Morrison and Davis, 1991; Naessens and Hopkins, 1996; Naessens and Howard, 1993; Saalmüller et al., 2005). Although the workshops were an effective way to complete characterization of many

* Corresponding author at: Department of Veterinary Microbiology, College of Veterinary Medicine, Seoul National University, Seoul, 151-742, Republic of Korea.
E-mail address: magic007@snu.ac.kr (K.T. Park).

http://dx.doi.org/10.1016/j.vetimm.2016.06.010
0165-2427/© 2016 Elsevier B.V. All rights reserved.

mAbs, limited resources and participants have curtailed efforts to continue convening international workshops, leaving many potentially useful mAb-defined molecules only partially characterized. The recent sequencing of the bovine genome and the development of mass spectrometry have provided information and technology needed for individual laboratories to continue the endeavor. Amino acid sequences identified from small quantities of immunoaffinity-purified proteins can be used to screen the genome databases of humans or other species to match sequences of known orthologous molecules. This proved useful recently in identifying the specificity of four mAb-defined molecules in cattle: CD26, CD50, the chaperone molecule gp96, and SLAMF9, the newest member of the signaling lymphocyte activation molecule family (Park et al., 2015b). Many potentially useful mAbs that recognize existing or new bovine molecules await complete characterization. Because of increased interest in determining the phylogenetic and functional similarities of ruminant monocytes to those characterized in mice and humans, the sets of partially characterized mAbs left over from the workshops were screened to determine if any of them recognize new molecules that might help clarify how closely ruminant monocyte ontogeny compares with ontogeny of monocytes in mice and humans. Any differences in ontogeny and expression of surface molecules on monocyte subsets could lead to misinterpretation of findings when comparing the ruminant (Italiani and Boraschi, 2014; Ziegler-Heitbrock and Hofer, 2013). Twelve mAbs were selected for further analysis. After analysis, the mAbs were used with mAbs specific for CD16, CD172a, and CD209 to extend information on what is known about the composition of monocyte subsets in ruminant blood.

2. Material and methods

2.1. Animals

Blood was obtained from steers, sheep, and goats being maintained on other projects. All animals were maintained according to Washington State University institutional animal care and use committee guidelines.

2.2. Monoclonal antibodies

The mAbs characterized in the present study were obtained from large sets of hybridomas developed from mice immunized with, bovine peripheral blood mononuclear cells (PBMCs) (BAQ, 159 hybridomas, prepared in 1986), a preparation of cells enriched for bovine monocyte and macrophages (MM, 65 hybridomas, prepared in 1991), a preparation of caprine macrophages (CAM, 73 hybridomas, prepared in 1992) and a preparation of bovine lymph node cells cultured to enrich for dendritic cells (DCs) and macrophages (LND, 89 hybridomas, prepared in 1998). FC was used as previously described to identify mAbs specific for molecules predominantly expressed on monocytes (Table 1) (Davis et al., 1995). Most of the hybridomas were cloned and set aside for later evaluation.

2.3. Generation of monocyte derived macrophages (Mo-MΦ)

For generation of Mo-MΦ, PBMCs were isolated as previously described (Koo et al., 2004). The PBMCs were re-suspended in RPMI containing 2% bovine calf serum (BCS) and cultured in tissue culture plates (5×10^6 cells/ml) at 37 °C/5% CO2 for two hours. After two hours, non-adherent cells were flushed out by washing with PBS. The adherent cells and remaining lymphocytes were cultured in complete RPMI-1640 (cRPMI, medium supplemented with 10 mM HEPES, 13% bovine calf serum, 2-mercaptoethanol, 1X GlutaMAX (Invitrogen), 100 units/ml of penicillin G and 100 μg/ml of streptomycin sulfate) at 37 °C/5% CO2 for seven days. On the fourth day, cultures were replenished with fresh cRPMI-1640.

2.4. SDS-PAGE, immunoprecipitation and western blotting

Mo-MΦ were harvested from the plates using 10 mM EDTA in PBS and pelleted by centrifugation. Pelleted cells were re-suspended in Pierce IP lysis buffer (0.025 M Tris, 0.15 M NaCl, 0.001 M EDTA, 1% NP-40, 5% glycerol; pH 7.4 containing protease and phosphatase inhibitors) for ten minutes, followed by centrifugation to harvest the cell lysate. The macrophage lysate was subjected to SDS-PAGE under reducing and non-reducing conditions using dithiothreitol (DTT) followed by western blotting to determine the kDa of the mAb-defined molecules (LND37A, LND68A and BAQ151A).

To purify the mAb-defined molecules, the lysates were subjected to immunoaffinity purification using mAbs cross-linked to protein A/G agarose beads according to the manufacturer's instructions. Briefly, the mAb protein A/G agarose beads were incubated with cell lysate for two hours at 4 °C then placed in a column. The column was then washed three times using IP lysis buffer. Molecules were eluted with elution buffer (pH 2.8). Eluates obtained from multiple cycles of immunoaffinity purification were concentrated using concentrator tubes (Pierce 10 kDa MWCO), and were then subjected to SDS-PAGE under non-reducing conditions. Protein bands from half of the gel were resolved by silver staining (SilverQuest™ Staining Kit, Invitrogen, CA) and the other half was subjected to western blotting to confirm the presence of the mAb-defined molecule at the expected predetermined kDa in the gel. The equivalent band in the silver stained gel, corresponding to the band in the western blot, was excised and submitted for analysis by mass spectrometry as previously described (Park et al., 2015b). Amino acid sequences obtained from the analyses were subjected to a search for sequence similarity against the bovine protein databases using the Mascot search engine. The minimum cutoff score was 50. At least two peptide sequences had to match sequences in the identified molecule before an apparent match was considered valid.

2.5. Flow cytometry

Single and multicolor FC were used to identify and characterize mAbs that recognize molecules expressed on monocytes and Mo-MΦ. All mAbs used in this study are listed in Table 1. The second step reagents used in the study were isotype specific goat anti-mouse immunoglobulins conjugated with FITC, PE, PE-Cy5.5 or Alexa Fluor 647 (Invitrogen, Life Technologies). In case of multicolor screening of mAbs of the same isotype (IgG1), Zenon labeling kits (Molecular Probes, Life Technologies) were used. Zenon-Fab fragments of IgG1 specific goat anti-mouse antibody conjugated with different fluorochromes (PE, or Alexa Fluor 647) were used, according to the manufacturer's instructions. For all cell preparations, labeling and staining were performed as previously described (Elnaggar et al., 2015; Park et al., 2015b). Labeled cells were fixed in 2% PBS-buffered formaldehyde and kept at 4 °C until analyzed by FC. Data were collected with a FACS Calibur flow cytometer equipped with a MAC computer and Cell Quest software (Becton Dickinson Immunocytometry Systems, San Jose, CA). The cells were gated according to a display of lymphocytes, monocytes, and granulocytes in SSC/FSC and color coded as follow: lymphocytes as red cells, monocytes and macrophages as blue cells and granulocytes as green cells. Cells displayed in blue contained large lymphocytes as well as monocytes. In some of the figures, a selective gate was placed on the monocyte zone to collect data on monocytes defined by expression of CD14 or CD163. FCS Express software (DeNovo

Download English Version:

https://daneshyari.com/en/article/2461294

Download Persian Version:

https://daneshyari.com/article/2461294

Daneshyari.com