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Short communication

Characterisation of monoclonal antibodies specific for hamster leukocyte differentiation molecules

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ABSTRACT

Flow cytometry was used to identify mAbs that recognize conserved epitopes on hamster leukocyte differentiation molecules (hLDM) and also to characterize mAbs developed against hLDM. Initial screening of mAbs developed against LDMs in other species yielded mAbs specific for the major histocompatibility (MHC) II molecule, CD4 and CD18. Screening of sets of mAbs developed against hLDM yielded 22 new mAbs, including additional mAbs to MHC II molecules and mAbs that recognize LDMs expressed on all leukocytes, granulocytes, all lymphocytes, all T cells, a subset of T cells, or on all B cells. Based on comparison of the pattern of expression of LDMs expressed on all hamster leukocytes with the patterns of expression of known LDMs in other species, as detected by flow cytometry (FC), four mAbs are predicted to recognize CD11a, CD44, and CD45. Cross comparison of mAbs specific for a subset of hamster T cells with a cross reactive mAb known to recognize CD4 in mice and one recognising CD8 revealed they recognize CD4. The characterization of these mAbs expands opportunities to use hamsters as an additional model species to investigate the mechanisms of immunopathogenesis of infectious diseases.

1. Introduction

The golden or Syrian hamster (*Mesocricetus auratus*) is used in biomedical research as a model for human and other animal diseases where the mouse is not appropriate. It is used as a model in multiple infectious diseases studies including Nipah virus (Wong et al., 2003), Hanta virus (Hammerbeck and Hooper, 2011), *Clostridium difficile* (Goulding et al., 2009) and safety testing of leptospirosis vaccines (Haake, 2006) and reviewed in Golden et al. (2015a,b). Of particular interest to us is its usefulness as a small animal model for research into malignant catarrhal fever in ruminants (Buxton et al., 1988; Jacoby et al., 1988; Russell et al., 2009). Hamsters offer an opportunity for adoptive cell transfer experiments to explore pathogenesis, as they are highly inbred (Campbell et al., 1996). This may be attributable to the current lineage being derived from three siblings caught in 1930 limiting genetic heterogeneity and functionality (Phillips et al., 1981).

The usefulness of the hamster as a small animal model for biomedical research has been constrained by a lack of immunological reagents to detect LDM differentially expressed on lymphoid cell subsets. Of the few monoclonal antibodies (mAbs) specific for hamster leukocyte differentiation molecules (hLDM) that have been developed, most are no longer available (Liu et al., 1991; Witte et al., 1985; Witte and Streilein, 1983a,b; Witte and Streilein, 1986). More recently the Washington State University Monoclonal Antibody Centre has addressed the growing need for reagents for use with this species. The reagents developed thus far have only been partially characterized.

The objective of the study presented here has been to complete the initial characterization of mAbs produced by the Centre and screen a selected set of commercially available mAbs for cross reactivity with hLDMs. These mAbs are available to the research community for further detailed characterisation.

2. Materials and methods

2.1. Animals

Spleen, lymph node and blood from disease-free Syrian hamsters of variable age and either sex were obtained from Harlan Laboratories (Loughborough, U.K.) and Charles River Laboratories, (San Diego, CA). Additional animals were obtained from a breeding-colony maintained at WSU. Ethical approval for the work was obtained from site ethical review committees at both WSU and the SVMS, University of Nottingham. The Nottingham ethical review was performed by the local animal welfare and ethical review body

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2.2. Antibodies used in this study

The antibodies used in this study are shown in Table 1. The mAbs were developed from mice immunized with hamster peripheral blood leukocytes (HAB), thymocytes (HAT), lymph node mononuclear cells (HAL), or a mixture of non-adherent and adherent mononuclear splenocytes (HASA) (Davis et al., 1987; McNees et al., 2009). Additional mAbs screened for cross reactivity to hLDMs were from commercial sources and the WSU Monoclonal Antibody Centre http://vmp.vetmed.wsu.edu/resources/monoclonal-antibody-center

2.3. Tissue collection and preparation

Blood was collected into 10% lithium heparin or acid citrate dextrose (ACD). Spleen (Spln) and mesenteric lymph nodes (MLN) were removed and placed into PBS. Mononuclear cell suspensions were prepared by either lymphoprep (Nycomed, Pharmacia, Oslo, Norway), or ammonium chloride – potassium cell lysis buffer (ACK, Gibco Life Sciences, U.K.), which retains both MNC and granulocytes. To obtain enough cells for each experiment, spleen and MLN MNCs were pooled.

2.4. Flow cytometry

Two methods were used to process cells for flow cytometry. Blood was collected in acid citrate dextrose (ACD) and used at 50 μl with 50 μl of mAb in tissue culture medium or in ascites (15 μg/ml) in 15 ml centrifuge tubes. Following 15 min of incubation on ice, the cells were sedimented by centrifugation and re-suspended in 10 ml of PBS containing 0.5% horse serum (PBSh). Following removal of the PBSh, the cells were labelled with R-phycoerythrin (PE) or fluorescein conjugated isotype specific second step goat anti-mouse IgG1, IgG2a, IgG2b, IgG3 or IgM antibody (Invitrogen, Carlsbad, CA, USA) alone or in combination to determine specificity. The rbc were lysed with Becton Dickinson fix/lyse solution (BD, Oxford, UK) re-sedimented and then re-suspended in 2% paraformaldehyde in PBS. When using the cross reactive mAbs that recognize epitopes conserved on hamster CD4 and CD8 T cells, a fluorescein conjugated goat mouse-absorbed anti-rat IgG second step was used for rat GK1.5 IgG2b mAb. A PE conjugated 341 IgG1 was used with HAB1A IgG1 labelled with fluorescein conjugated anti-IgG1 Zenon reagent (Invitrogen, Carlsbad, CA, USA).

For the second method, cells in RPMI-1640 with 2% FBS or PBS with 2% FBS were distributed into 96 well culture plates (2×10^5 to 1×10^6 cells per well). 50 μl of appropriately diluted primary antibodies were added to the cells. Following incubation for 30 min (4 °C) the plates were centrifuged at 2000 rpm for 2 min and the supernatant removed. The cells were washed twice and incubated alone or in combination with isotype specific goat anti-mouse IgG or goat anti-rat IgG antibodies conjugated with fluorescein, PE or allophycocyanine (APC). In some experiments whole blood was incubated with mAbs and then the rbc were removed using 1 x BD FACSTM lysing solution before continuing with the labelling process. A Becton Dickinson FACS Calibur (Immunocytometry Systems, San Jose, CA, USA) (WSU) and Beckman Coulter EPICS Altra, FC500 and MoFlo XDP (School of Molecular Medical Sciences in the Queen's Medical Centre, Nottingham, UK) were used to collect data. Data were analysed with FCS Express, LA, USA and the Beckman Coulter programs.

For flow cytometric analysis (FC), three electronic gates were used to identify and colour code regions of a dot plot display in side (SSC) vs forward light scatter (FSC) containing lymphocytes (L, orange), predominantly monocytes (M, blue), and granulocytes

Table 1
Monoclonal antibodies (WSU Monoclonal Antibody Centre) and Specificities.

mAb	Ig isotype	Putative specificity[1]	Specificity and% of cells[2]
H42A	IgG2a	MHC II	MHC II, 48%
BAQ30A	IgG1	CD18	CD18, 100%
HAL4A	IgG3	MHC class II	MHC class II, 50%
HAL16A	IgG1	MHC class II	MHC class II, 50%
HAB2A	IgG1	T	33%–43% (CD4 included)
HAL26A	IgG1	T	42%–63% (CD4 included)
HAT19A	G2a	T	39–53% (CD4 included)
HAT24A	IgG1	T	53%–73%
HAB1A	IgG1	T subpopulation	12–44% (CD4)
HAL36A	IgG2a	T subpopulation	16–42% (CD4)
HAL9A	IgG1	B	n.d.
HAL11A	IgG1	B	n.d.
HAL14A	IgG2b	B	B 23%
HAL17A	IgG2a	B	n.d.
HASA7A	IgG1	B	34–49% not CD4
HAB6B	IgG2a	Pan lymphocyte (+monocyte subset?)	n.d.
HASA18A	IgM	Pan lymphocyte (+monocyte subset?)	n.d.
HASA25A	IgG1	CD45 predicted	n.d.
HAT13A	IgG2b	CD45 predicted	CD45
HAT7A	IgG2a	CD44 predicted	n.d.
HAT16A	IgG2b	CD11a predicted	n.d.
HAB3A	IgG1	Pan leukocyte	>95% incl CD4 and CD?
HASA26B	IgG1	Granulocyte +	n.d.

Legend:
[1] Based on labelling characteristics of lymphocyte, monocyte and granulocyte-enriched fractions of whole blood leukocytes and 2-colour FC comparison with MHC class II positive and negative fractions of the leukocytes.
[2] Proposed specificities based on two colour comparisons of MNC (PBMC or spleen/MLN MNC) labelling by the mAbs compared to each other and a defined CD-specific mAb (GK1.5 anti-CD4). For the frequency ranges of the phenotyped cells, six different MNC samples from different hamsters were used for the analyses, but not all antibodies were tested at the same time (n = 3 or 4). This is why the% frequencies of HAB2A, HAL 26A and HAT19A have different ranges, in spite of recognising the same molecule. Nd = not determined. These mabs are listed as they are available for further characterisation by the research community.