Supplemental information

Immunological reagents for non-murine rodents. The use of Syrian hamsters to model biological diseases is not unique to hantaviruses. Hamsters have been considered a valuable model to study the disease caused by many human pathogens including Toxocara canis (1), Nipah virus (2, 3), measles virus (4), prion diseases (5), Leishmania (6) Yellow fever virus (7), SARS (8, 9), West Nile virus (10, 11), encephalitis viruses (12-15) and Ebola virus (16), due to the numerous similarities between the disease manisfested in hamsters and the natural course of human disease pathogenesis. However, the hamster model is unique from its mouse and rat counterparts in that, despite pioneering studies in the 1980s identifying lymphocyte subsets in the Syrian hamster (17-21) and the cloning of multiple hamster cytokine genes in 2000 (22), there have been relatively few advances in the development and characterization of commercially available reagents to study immunological mechanisms in the hamster. The identification of anti-mouse CD4 (17, 23) and anti-rat CD8 antibodies that cross-react with orthologous proteins expressed on hamster CD4 and CD8 T cells have made it possible to global T cell responses to ANDV infection in hamsters by flow cytometry)(24, 25). In these studies, T cell activation and accumulation in the lung correlated with the onset of disease in hamsters but the depletion of CD4 and/or CD8 T cell subsets in hamsters did not prevent disease. However, the majority of mouse and rat specific for other T cell specific surface molecules (i.e. activation markers, adhesion markers, inhibitory ligands, memory markers) or intracellular cytokines do not cross react with their hamster ortholog, or have not been identified, making detailed analysis of T cell responses in hamsters difficult. More recently, an antibody specific for the hamster CD3 antigen has been developed and used to deplete hamster T cells in a model of tumor immunity (26) but this antibody is not commercially available. With the exception of one antibody that identifies the MARCO scavenger receptor on Syrian hamster alveolar macrophages(27), flow cytometry antibodies specific for most hamster immune cell types (e.g. neutrophils, monocytes, NK cells, dendritic cells) are scarce. A handful of hamster-specific monoclonal antibodies, or cross-reactive monoclonal antibodies, to several common lymphocyte antigens (CD18, CD4, CD8, MHC I) as well as three antibodies that more broadly identify hamster immune cells (T cell, B cell and lymphocyte) are available from Kingfisher Biotech, Inc. Along with flow cytometry assays, the use of mouse and rat assays that rely on conformational protein epitopes, such as ELISA and Luminex assays, have been problematic in their ability to accurately recognize hamster proteins. While a few commercially available mouse and rat ELISA and Luminex assays have been found to suitably cross-react with hamster samples, many produce uncharacteristically abnormal measurements or fail to cross-react altogether (28). Notwithstanding, within the last several years a variety of hamster-specific cytokine ELISA assays have recently become available by the online company MyBioSource. Results using these kits have yet to be reported in the literature but we have found that hamster protein concentrations detected by kits from various tissue sources (e.g. serum, lung homogenates, BAL fluid) to fall within the acceptable limits of the standards. Antibodies that cross-react with linear hamster protein eptitopes have been more successful in identifying the expression of numerous proteins by western blot analysis such as caspases, matrixmetalloproteinases, autophagy pathway member proteins such as ubiquitin and proteins involved in inflammatory cytokine responses such as NF-κB, JAK2 and STAT3 (29-35). Similarly, expanded sets of PCR primers for over 70 hamster proteins have been published (22, 28, 36) and used in hamster models of disease pathogenesis and oncology and the recent sequencing, annotation and analysis of the Syrian hamster transcriptome(37) should facilitate the expansion of a new generation of resources to study the hamster model. A list of reagents compatible with the Syrian hamster, including cross reactive flow cytometry and western blot antibodies, cross-reactive ELISA and Luminex assays, cytokines and PCR primers, is shown in **Table S1**.

Table S1. Immunological reagents specific for, or known to cross-react with, the Syrian hamster.

Western Blot Antibodies			
Antigen	***Tissue(s) Analyzed***	***Antigen***	***Tissue(s) Analyzed***
Akt (phospho)	Heart(1)	**MMP-9**	Buccal pouch(2, 3)
Apaf-1	Buccal pouch(2)	**NFκB-p50**	Buccal pouch(2)
Bax	Buccal pouch(2, 3)	**NF-κB-p65**	Buccal pouch(2)
Bcl-2	Buccal pouch(2, 3) Liver(4)	**p21^{waf-1}**	Buccal pouch(2, 3)
Bcl-xL	Liver(4)	**p53**	Buccal pouch(2, 3)
c-fos	Liver(4)	**p65**	Liver(4)
c-jun	Liver(4)	**p65** (phospho)	Liver(4)
Caspase-2L	Buccal pouch(2, 3)	**PARP**	Buccal pouch(3) Liver(4)
Caspase-3	Buccal pouch(2, 3) Liver(4) Endometrium(5)	**PARP** (cleaved)	Liver(4)
Caspase-6	Buccal pouch(2, 3)	**Procaspase 3**	Liver(4)
Caspase-8	Buccal pouch(2, 3) Liver(4)	**Procaspase 8**	Liver(4)
Caspase-9	Buccal pouch(2, 3) Liver(4)	**Procaspase 9**	Liver(4)
Cathepsin D	Heart(1)	**Rab7**	Heart(1)
COX-2	Buccal pouch(6) Liver(4)	**RECK**	Buccal pouch(3)
Cytochrome C	Buccal pouch(2, 3)	**STAT-3**	Liver(4)
ERK (phospho)	Heart(1)	**STAT-3** (phospho)	Liver(4) Heart(1)
Fas	Buccal pouch(2, 3)	**Survivin** (C)	Buccal pouch(3)
HIF-1	Buccal pouch(2, 3)	**Survinin** (N)	Buccal pouch(3)
ICAM-1	Lung(7)	**TIMP-2**	Buccal pouch(3)
IκB	Buccal pouch(2)	**TNFα**	Heart(1)
iNOS	Liver(4)	**TRAF1**	Liver(4)
JAK2	Liver(4)	**Ubiquitin**	Heart(1)
Mcl-1	Buccal pouch(3)	**VCAM-1**	Lung(7)
MMP	Heart(1)	**VE-cadherin**	Lung(7)
MMP-2	Buccal pouch(2) (3)	**VEGF**	Buccal pouch(2) (3) Endometrium(5)

Immunohistochemistry Antibodies			
Antigen	***Tissue(s) Analyzed***	***Antigen***	***Tissue(s) Analyzed***
Akt (phospho)	Heart(1)	**iNOS**	Liver(4)
Bax	Buccal pouch(2, 3, 8)	**MMP-2**	Uterus(9)
Bcl-2	Buccal pouch(2, 3, 8)	**p53**	Buccal pouch(8)
Caspase-3	Buccal pouch(2)	**p65**	Liver(4)
Cathepsin D	Heart(1)	**MARCO**	Lung(10)
COX-2	Buccal pouch(6)	**RECK**	Buccal pouch(2)
factorVIII/von	Buccal pouch(6)	**Stat3** (phospho)	Heart(1)

Willebrand factor	Heart(1)		
Flk-1	Vascular endothelial cells(11) Uterus(9)	**TUNEL**	Heart(1)
FLT-1	Uterus(9)	**VEGF**	Buccal pouch(8) Vascular endothelial cells(11) Uterus(9)

Flow Cytometry / In Vivo Depletion Antibodies

Antigen	*Tissue(s) Analyzed*	*Antigen*	*Tissue(s) Analyzed*
Annexin V (mammalian)	Spleen/PBMC/lung(12)	**FoxP3** (FJK-16s; mouse/rat)	Spleen/lung (Hammerbeck unpublished)
BrdU (3D4; human)	Spleen/PBMC/lung(12)	**MHC II** (14-4-4S; mouse I-E^k/rat RT1D)	Spleen/PBMC/lung(12) Spleen(13) (14)
CD3 (4F11; mouse)	Lymphoid/nonlymphoid tissue(15)	**MARCO** (PAL-1; hamster)	Lung/BAL(10)
CD4 (GK1.5; mouse)	Spleen/PBMC/lung(12) Spleen(16) (14)	**TNFα** (MP6-XT22; mouse)	Spleen (Hammerbeck unpublished)
CD8β (341; rat)	Spleen/PBMC/lung(12) Spleen(14)		

Recombinant Cytokines

Cytokine	*Reference*	*Cytokine*	*Reference*
G-CSF (human)	Miyata(1)	**Platelet-activating factor (PAF)**	Kim(17)
GM-CSF (human)	Parviainen(18)	**TNFα** (mouse)	Falcinelli et.al.(7)
IL-2 (mouse)	Sobotkova(19)	**$VEGF_{165}$** (human)	Kim(17) Aramoto(20)
IL-12 (mouse)	Bortolanza(21)		

ELISA Kits

Analyte	*Tissue(s) Analyzed*	*Analyte*	*Tissue(s) Analyzed*
c-fos	Buccal pouch(8)	**PGE2**	Buccal pouch(8)
COX-2	Buccal pouch(8)	**TGF-β** (rat; marginal)	Plasma(22)
Caspase-3, -9	Buccal pouch(8)	**IL-10** (rat; marginal)	Plasma(22)
VEGF (human)	Buccal pouch(8)	**IL-1β** (mouse; marginal)	Plasma(22)

Lumiex Panels

Analyte	*Tissue(s) Analyzed*	*Analyte*	*Tissue(s) Analyzed*
GM-CSF (rat)	Plasma(22)	**MIG**	Plasma(22)
Leptin (rat)	Plasma(22)	**IL-13**	Plasma(22)
GRO/KC (rat)	Plasma(22)	**Eotaxin** (mouse; marginal)	Plasma(22)
IL-1α (rat)	Plasma(22)	**MIP-1β** (mouse; marginal)	Plasma(22)
RANTES (rat; marginal)	Plasma(22)	**IL-12p40** (mouse; marginal)	Plasma(22)
IL-10 (rat; marginal)	Plasma(22)		

PCR Primers

Protein	*Tissue(s) Analyzed*	*Protein*	*Tissue(s) Analyzed*
Apaf-1	Buccal pouch(3)	**IL-21**	Spleen, liver, lung(22)
β2 microglobulin	Spleen, liver, lung(22)	**IL-2Rα**	Spleen, liver, lung(22)
β-actin	Spleen, liver, lung(22)	**IL-6 signal transducer**	Spleen, liver, lung
Bad	Buccal pouch(3)	**iNOS**	Spleen, liver, lung(22) Spleen(23)

Bax	Buccal pouch(2) (3)	**IP-10**	Spleen, liver, lung(22)
Bcl-2	Buccal pouch(2) (3) Spleen, liver, lung(22)	**IRF1**	Spleen, liver, lung(22)
Bcl-2 associated protein	Spleen, liver, lung(22)	**IRF2**	Spleen, liver, lung(22)
Bcl-xL	Buccal pouch(3)	**Junction adhesion molecule**	Spleen, liver, lung(22)
Bid	Buccal pouch(3)	**MMP-2**	Spleen, liver, lung(22) Buccal pouch(2) (3)
CCL20/MIP-3a	Spleen, liver, lung(22)	**MMP-9**	Buccal pouch(2) (3)
CCL17	Spleen, liver, lung(22)	**MHC II alpha chain**	Spleen, liver, lung(22)
CCL22	Spleen, liver, lung(22)	**Muc1**	Spleen, liver, lung(22)
CD83	Spleen, liver, lung(22)	**Myxovirus resistance protein-2**	Spleen, liver, lung(22)
c-FLIP	Buccal pouch(3)	**NOS2**	Spleen, liver, lung(22)
Claudulin-1	Spleen, liver, lung(22)	**Occuldin**	Spleen, liver, lung(22)
Complement C3	Spleen, liver, lung(22)	**p21**	Buccal pouch(3)
Complement C5	Spleen, liver, lung(22)	**P75 TNF membrane receptor**	Spleen, liver, lung(22)
Complement protein C1q	Spleen, liver, lung(22)	**PCNA**	Buccal pouch(3)
Cyclin D1	Buccal pouch(3)	**PECAM**	Spleen, liver, lung(22)
Cyt-C	Buccal pouch(3)	**PIGF**	Buccal pouch(3)
E-cadherin	Spleen, liver, lung(22)	**Protein kinase R**	Spleen, liver, lung(22)
Fas-L	Buccal pouch(3)	**Ribosomal Protein L18**	Spleen, liver, lung(22)
Fibrinogen	Spleen, liver, lung(22)	**STAT 1**	Spleen, liver, lung(22)
FoxP3	Spleen, liver, lung(22)	**STAT1b**	Spleen, liver, lung(22)
GST-P	Buccal pouch(3)	**STAT2**	Spleen, liver, lung(22)
Hypoxanthine phosphoribosyltransfe rase	Spleen, liver, lung(22)	**Tight junction protein 2**	Spleen, liver, lung(22)
ICAM-1	Spleen, liver, lung(22)	**TIMP-2**	Spleen, liver, lung(22) Buccal pouch(3)
IFNα inducible protein	Spleen, liver, lung(22)	**TGF-β**	Spleen(24) (23)
IFNγ	Spleen, liver, lung(22) Spleen(24) (23)	**TGF-β1**	Spleen, liver, lung(22)
IL-1β	Spleen, liver, lung(22)	**TGF-β2**	Spleen, liver, lung(22)
IL-2	Spleen, liver, lung(22) Spleen(24) (23)	**TGF-β3**	Spleen, liver, lung(22)
IL-4	Spleen, liver, lung(22) Spleen(24) (23)	**TGF-β1R**	Spleen, liver, lung(22)
IL-6	Spleen, liver, lung(22)	**TNFα**	Spleen, liver, lung(22) Spleen(24) (23)
IL-10	Spleen, liver, lung(22) Spleen(24) (23)	**VEGF**	Spleen, liver, lung(22) Buccal pouch(2) (3)
IL-12 p35	Spleen, liver, lung(22)	**VEGFR1**	Buccal pouch(3)
IL-20p40	Spleen, liver, lung(22) Spleen	**VEGFR2**	Buccal pouch(3)

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