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Screening monoclonal antibodies for cross-reactivity in the ferret model of influenza infection

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Abstract

Influenza virus infections carry a high public health cost, and pandemics are potentially catastrophic. Though the ferret is generally regarded as the best model for human influenza, no reagents are available for the analysis of cellular immunity. We thus screened monoclonal antibodies (mAbs) made for identifying immune cells in other species to see if any were cross-reactive. Flow cytometric analysis of lymphocytes isolated from blood, spleen, and lung of normal and virus-infected ferrets indicated that several mouse mAbs bound to the corresponding antigens in ferrets. Typing bronchoalveolar lavage populations from pneumonic ferrets with mAb to human CD8 showed the massive CD8+ T cell enrichment characteristic of this infection in mice. The availability of this, and several other mAbs that showed cross-reactivity, should allow us to begin the dissection of cell-mediated immunity in the ferret, which, at least from these early results, looks similar to the situation in mice.

Keywords

Ferret; influenza; CD8+ T lymphocyte; mice; flow cytometry

1. Introduction

The influenza A viruses are segmented, negative strand RNA viruses of the *Orthomyxoviridae* family (Wright and Webster, 2001). The natural reservoir is wild birds, yet many species are susceptible to infection. In humans, influenza infects the respiratory epithelium through attachment of its surface hemagglutinin (HA) to $\alpha 2, 6$ sialic acid receptors on the cell surface (Cabezas et al 1980 and Daniels et al, 1984). Neuraminidase (NA) on the viral surface facilitates release of the virion from the cell. Individual strains are distinguished by serological differences in their HA and NA proteins.

Several strains of influenza virus circulate throughout the human population, manifesting in seasonal epidemics. Protection is mediated by neutralizing antibody against the viral HA and NA. However, antigenic drift in the surface HA and NA allows the virus to be maintained in the population and potentially escape antibody responses upon re-infection. Moreover, antigenic shift, by gene swapping between viruses during co-infection, can allow new strains to emerge and wreak havoc on the human population. As a result, influenza infection carries a significant public health cost. Estimates suggest over 200,000 hospitalizations and 30,000 deaths are attributable to influenza every year in the United States alone (Thompson et al,

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2003 and 2004). The extremely high mortality associated with very pathogenic strains of H5N1 avian influenza causing albeit rare infections in the human population (World Health Organization, website) has aroused serious concerns about the possible emergence of another catastrophic pandemic, similar in devastation to the Spanish Flu of 1918-1920 (Webby and Webster, 2003).

The ferret is considered to develop a disease process that is most like human influenza infection (Maher and DeStefano, 2004), though the analysis of T cell-mediated immunity has largely been done in the mouse (Thomas et al., 2006). One reason for this is that ferret studies are hampered by a severe lack of immunological reagents. The ferret is a mustelid, and previous studies have identified cross-reactive monoclonal antibodies (mAbs) that bind mustelid proteins in mink (Aasted 1989, Danilenko et al., 1992, Jacobsen et al., 1993, Chen et al., 1997, Sager et al., 1997, Brodersen et al., 1998, Pedersen et al., 2002, Jensen et al., 2003, Saalmuller et al., 2005). These antibodies were originally generated against human, canine, ovine, and bovine cells.

Aiming to expand the reagent repertoire, we used flow cytometry to test a panel of readily-available mouse mAbs against cells from normal and influenza virus-infected ferrets. When compared to mouse splenocytes, we found several monoclonal antibodies that cross-reacted with proteins on ferret lymphocytes recovered from blood, spleen, and bronchoalveolar lavage (BAL) of the infected lung. Some of these cross-reactivities have been found previously for mink, but to our knowledge, not for the ferret proteins. This allowed us to show that, at least numerically, the CD8+ T cell response is comparable in ferrets and mice. This allows us to begin the analysis of cell-mediated immunity in the ferret.

2. Materials and Methods

2.1. Animals

Ferrets were purchased from Marshall Farms (North Rose, NY) and housed under pathogen-free condition at SJCRH. All studies were conducted under applicable laws and guidelines and after approval from the St. Jude Children's Research Hospital Animal Care and Use Committee.

2.2 Viruses and infection

In initial experiments, ferrets were infected with A/SW/MO/22454/06 (H2N3), and PBMCs or memory splenocytes were analyzed several weeks later. In subsequent experiments, ferrets were primed intranasally with either A/SW/NC/18161/02 (H1N1) or A/Mallard/Alberta/79/03 (H2N3). Most ferrets were then challenged with A/Wuhan/359/95 (H3N2) > 1 month later, while one ferret from each group remained unchallenged. At day 7 after challenge, spleens and BAL were collected for analysis.

2.2. Flow cytometry

Because of their size, ferret spleens were first chopped into small pieces, then manually disrupted between the frosted ends of two glass slides in sterile PBS containing 2% FBS (2% PBS), followed by red cell lysis. Bronchoalveolar lavage (BAL) was collected with 10ml Hank's buffered saline solution. Cells were washed in 2% PBS and stained with a multitude of fluorescently-labeled antibodies (Table 1). PBMCs were isolated from ferret blood by spinning over a FICOLL gradient at $400 \times g$ for 20 minutes, then washing the mononuclear cell layer in PBS. For intracellular cytokine staining (ICS), cells were cultured in round bottom 96 well plates and treated with 1mg/ml of PMA/ionomycin and brefeldin A in RPMI +10% FCS for 5 hours at 37°C. After in vitro stimulation, the cells were washed, fixed, and permeabilized according to the manufacturer's protocol (BD PharMingen Cytofix/Cytoperm

kit). All cells were then stained with the antibodies listed in Table 1. Samples were run on a BD FACScalibur, and data were analyzed using FlowJo software (Tree Star, San Carlos, CA).

3. Results

3.1. Antibodies previously shown to recognize mink antigens also recognize antigens on PBMCs from influenza-immunized ferrets

The ferret is an accepted model of influenza infection. Because so few immunological reagents exist for ferrets, the scope of experiments that can be performed with this model is unfortunately limited. We therefore decided to test a series of fluorescently-labeled antibodies that we frequently use to study murine immune responses to influenza infection in the ferret model.

Ferrets that had been immunized with A/SW/MO/22454/06 were bled to obtain PBMCs. Mononuclear cells were isolated over a FICOLL gradient and washed in PBS. The anti-human CD8 clone OKT8 has been shown to stain mink CD8⁺ T cells (Aasted, 1989), so we hoped that it would also stain ferret CD8⁺ T cells. It did (Fig. 1A), giving us a positive control and, importantly, a CD8⁺ T cell marker for ferrets. We also saw positive staining with another antibody that had been shown to cross-react with mink, anti-mouse CD11b (Dannilenko, et al., 1992) (Fig. 1B). Among the panel of antibodies that we tested, we also saw clear, positive staining with anti-mouse CD44 and anti-mouse Thy 1.1, which corresponded to control staining of mouse splenocytes (Fig. 1C-D). We additionally saw low level positive staining of CD43, LFA-1, and CD103 (Fig. 1E-G). In one experiment we also saw very low level staining with anti-mouse CD4 (clone RM4-5), anti-CD8 (clone 53-6.7), and anti-CD19, but these results did not consistently repeat (data not shown). This is likely a result of allelic variation between animals (Johnson and Porter, 2000, Yan, et al., 2002). The entire panel of antibodies that we tested over two separate experiments with blood samples is shown in Table I. Our observations suggest that results previously obtained in mink studies are likely to correlate with results obtained from ferrets. In addition, the positive staining we observed with CD44 and Thy1.1 showed that there is strong potential to identify new ferret reagents using commercially available fluorescently-labeled anti-mouse antibodies.

3.2. Cross-reactive anti-mouse antibodies recognize surface antigens on cells from ferret BAL and spleens after influenza infection

Encouraged by the above results, we analyzed phenotypic characteristics of the immune response in ferrets after influenza infection. A total of twelve ferrets were primed with influenza A virus, then eleven received challenged with heterologous influenza A viruses as described in the Materials and Methods. At day 7 post-challenge, the ferrets were sacrificed and BAL and spleens were collected.

Once again, the OKT8, CD44, CD11b, and Thy1.1 antibodies were obviously positive, in both the BAL (Fig. 2) and spleen (data not shown). We also noted positive staining with CD25, CD43, Ly6C, and LFA-1 in both the challenged ferrets (Fig. 2D-G) and the unchallenged ferret (data not shown). Background was minimal in unstained BAL samples (Fig. 2H).

The percent of BAL cells staining positive for each antigen is shown in graph form in Figure 3. Of note is that similar percentages of cells stained positive for OKT8 and Thy1.1, regardless of the virus that was used for the primary infection. Allelic variation between these outbred animals was evident in the Thy1.1 staining, as the percentage of OKT8⁺ CD8 T cells that were Thy1.1⁺ ranged from 30.87% to 97.1%, with an average of $64.93 \pm 20.35\%$ (Fig. 2C). Since most peripheral CD8⁺ T cells express Thy1.1, this suggests that the pulmonary infiltrate is heavily biased toward CD8⁺ T cells in infected ferrets. Comparisons of the two viruses used in the primary infection indicated slight differences in OKT8⁺ and Thy1.1⁺ cell populations

in the BAL and spleen after challenge, but these differences were not significant (Fig. 3). These data indicate that lymphocytes dominate the pulmonary infiltrate during acute infection, paralleling the situation in mice. Also similar to the mouse lung after infection, CD11b⁺ APCs ranked second in terms of inflammation. We also noted that our Ly6C⁺ cells also exhibited low level CD11b expression. The expression levels of the antigens that we tested were similar regardless of the virus used for primary infection. In addition, we were still able to detect resident populations of positive cells for multiple antigens in the ferret that was primed with H2N3, but did not receive a secondary challenge. Staining patterns in the spleen were similar to what was seen in the BAL

3.3. Cross-reactive anti-mouse antibodies recognize intracellular cytokines within cells from ferret BAL and spleens after influenza infection

We also performed intracellular cytokine staining (ICS) on cells from ferret BAL and spleens. Two antibodies against bovine IFN- γ and IL-4 that have been shown previously to cross-react with mink (Pedersen et al., 2002 and Jensen et al., 2003) also provided positive staining in our ICS of cells from ferret BAL and spleen (Fig. 4A and C). This was also the case for anti-mouse IFN- γ (clone XMG1.2) and anti-mouse TNF (clone MP6-XT22) in the BAL and spleen (Fig. 4B and D). Evidence of minimal staining was found for an anti-mouse IL-2, but this was not intense enough to be considered positive. Furthermore, the virus used in the primary infection did not influence differential antiviral cytokine production when the cells were restimulated with PMA + ionomycin. The indications are, though, that it is now possible to detect antiviral cytokine production in cells recovered from long-term- and acutely-infected ferrets.

4. Discussion

In this study, we have confirmed that several readily-available fluorescently-labeled antibodies with a variety of host specificities will cross-react with ferret antigens. Some of these antibodies have been tested previously and were shown to cross-react with antigen on mink cells (summarized by Broderick et al., 1998). To our knowledge, the current study represents the first description of cross-reactivity with these antibodies in ferrets, while also identifying several new antibodies that recognize ferret antigens.

Of particular interest is the observation that the anti-human CD8 clone OKT8 will recognize ferret CD8⁺ T cells. As in other respiratory virus infections, the CD8⁺ T cell has been shown to be important in the resolution of influenza virus infection (Doherty et al., 1997 and Thomas et al., 2006). Moreover, CD8⁺ T cells can also cause significant immunopathology in severe infections (Moskophidis and Kioussis, 1998). This is especially relevant given the current situation with highly pathogenic H5N1 avian influenza infections, which may induce debilitating immunopathology through hypercytokinemia, or the “cytokine storm” (Seo et al., 2002, Cheung et al., 2002, Lipatov et al., 2005, Chan et al., 2005). As a mutated H5N1 avian influenza strain could emerge as the next great human pandemic, it is important to have a CD8⁺ T cell-specific antibody for use in the ferret model.

Of particular substance is the sizeable infiltrate of OKT8⁺ and Thy1.1⁺ cells that we observed in the BAL populations recovered from these outbred ferrets. Thy1.1 is expressed on T cells in the mouse, but its expression is variable between species (Haeryfar and Hoskin, 2004). The level of Thy1.1 staining seemed low in samples from some ferrets, indicating that, as in the mouse, there may be allelic forms of this molecule (Johnson and Porter, 2000, Yan, et al., 2002). Such diversity also may have contributed to the variable staining profiles with anti-mouse CD4, CD8, and CD19 antibodies. However, many of the antibodies we tested for cross-reactivity exhibited populations that were clearly positive in more than one ferret.

Not surprisingly, our data demonstrated that a high percentage of these Thy1.1+ cells were also CD8+, due to their positive staining with OKT8. Impressively, the overwhelming majority of CD8+ T cells were CD44+, and a robust CD11b+ population was also observed in the BAL of infected ferrets. These observations are noteworthy because they illuminate a strong parallel to the mouse model of influenza, indicating that the pulmonary cellular infiltrate in the ferret is similar in at least some respects to that in the mouse, and thus fortifies observations and conclusions collected in mouse studies.

Equally important is the identification of multiple anti-cytokine antibodies that can be used to identify activated ferret lymphocytes. Two antibodies that previously exhibited cross-reactivity with mink cells, anti-bovine IFN- γ and IL-4 (Pedersen et al., 2002 and Jensen et al., 2003), were also cross-reactive for ferret cells in our study. In addition, we noted that anti-mouse IFN- γ and TNF also exhibited positive staining in our study. One point of potential concern is that we could not consistently distinguish a clear OKT8+ population after the ICS incubation, so we could not always identify OKT8+/IFN- γ + or OKT8+/TNF+ populations. This may possibly be due to downregulation of the CD8:TCR complex after activation (Robbins and McMichael, 1991), which of course not only leads to loss of surface CD8:TCR, but may also result in conformational changes and even degradation after internalization. Because we used PMA/ionomycin, a strong mitogen that stimulates all CD8+ T cells intensely and nonspecifically, there is surely a significant loss of CD8 on the T cells during the ICS incubation. Nevertheless, the identification of IFN- γ -, TNF-, and IL-4-specific antibodies for use in the ferret greatly expands the number and complexity of questions that can be asked using this model of influenza infection.

In addition to confirming the cross-reactivity in ferrets of several antibodies that had been previously described in mink, we also identified a handful of cross-reactive antibodies that had not, to our knowledge, been described prior to our study. Basic immunology questions about CD8+ T cells can now be investigated with the ferret model. Furthermore, these newly-identified reagents will allow for studies of monocytes (Ly6C with CD11b), adhesion (LFA-1), and T cell activation (CD44, CD25, CD43), which will expand knowledge of basic immunology as well as pathogenesis.

Severe human influenza infections, such as the 1918 pandemic and the current H5N1 crisis, are associated with a massive pulmonary cellular infiltrate (Hsieh et al., 2006), much like the situation found in the mouse after infection with highly virulent variants of the PR8 virus. The availability of the reagents described here will certainly facilitate the analysis in the more comparable (to humans) ferret model, and allow better insights into the validity, or otherwise, of the much more detailed results that can be obtained from mouse model systems.

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References

- Aasted B. Mink infected with Aleutian Disease Virus have an elevated level of CD8-positive T lymphocytes. *Vet Immunol Immunopath* 1989;20:375–385.
- Brodersen R, Bijlsma F, Gori K, Jensen KT, Chen W, Dominguez J, Haverson K, Moore PF, Saalmuller A, Sachs D, Slierendrecht WJ, Stokes C, Vainio O, Zuckerman F, Aasted B. Analysis of the immunological cross reactivities of 213 well characterized monoclonal antibodies with specificities against various leucocyte surface antigens of human and 11 animal species. *Vet Immunol Immunopath* 1998;64:1–13.

- Cabezas JA, Calvo P, Eid P, Martin J, Perez E, Reglero A, Hannoun C. Neuraminidase from influenza A virus A (H3N2): specificity towards several substrates and procedure of activity determination. *Biochim Biophys Acta* 1980;616:228–238.
- Campbell DG, Gagnon J, Reid K, Williams AF. Rat brain Thy-1 glycoprotein. The amino acid sequence, disulphide bonds and an unusual hydrophobic region. *Biochem J* 1981;195:15–30. [PubMed: 6118137]
- Chan MCW, Cheung CY, Chui WH, Tsao SW, Nicholls JM, Chan YO, Chan R, Long HT, Poon L, Guan Y, Peiris JSM. Proinflammatory cytokine responses induced by influenza A (H5N1) viruses in primary human alveolar and bronchial epithelial cells. *Resp Res* 2005;6:135–147.
- Chen W, Pedersen M, Gram-Nielsen S, Aasted B. Production and characterization of monoclonal antibodies against mink leukocytes. *Vet Immunol Immunopath* 1997;60:161–170.
- Cheung CY, Poon L, Lau AS, Luk W, Lau YL, Shortridge KF, Gordon S, Guan Y, Peiris JSM. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease. *Lancet* 2002;360:1831–1837. [PubMed: 12480361]
- Daniels RS, Douglas AR, Skehel JJ, Wiley DC, Naeve CW, Webster RG, Rogers GN, Paulson JC. Antigenic analyses of influenza virus hemagglutinins with different receptor-binding specificities. *Virology* 1984;138:174–177. [PubMed: 6208680]
- Danilenko DM, Moore PF, Rossitto PV. Canine leukocyte cell adhesion molecules (LeuCAMs): characterization of the CD11/CD18 family. *Tissue Antigens* 1992;40:13–21. [PubMed: 1359668]
- Doherty PC, Topham DJ, Tripp RA, Cardin RD, Brooks JW, Stevenson PG. Effector CD4+ and CD8+ T cell mechanisms in the control of respiratory virus infections. *Immunol Rev* 1997;159:105–117. [PubMed: 9416506]
- Haeryfar SM, Hoskin DW. Thy-1: more than a mouse pan-T cell marker. *J Immunol* 2004;173:3581–3588. [PubMed: 15356100]
- Hsieh YC, Wu TZ, Liu DP, Shao PL, Chang LY, Lu CY, Lee CY, Huang FY, Huang LM. Influenza pandemics: past, present and future. *J Formos Med Assoc* 2006;105:1–6. [PubMed: 16440064]
- Jacobsen CN, Aasted B, Broe MK, Petersen JL. Reactivities of 20 anti-human monoclonal antibodies with leucocytes from ten different animal species. *Vet Immunol Immunopathol* 1993;39:461–466. [PubMed: 8116221]
- Jensen PV, Casteluiz Y, Aasted B. Cytokine profiles in adult mink infected with Aleutian Mink Disease Parvovirus. *J Virol* 2003;77:7444–7451. [PubMed: 12805443]
- Keating R, Yue W, Rutigliano JA, So J, Olivas E, Thomas PG, Doherty PC. Virus-specific CD8+ T cells in the liver: armed and ready to kill. *J Immunol* 2007;178:2737–2745. [PubMed: 17312116]
- Lipatov AS, Andreansky S, Webby RJ, Hulse DJ, Reh JE, Krauss S, Perez DR, Doherty PC, Webster RG, Sangster MY. Pathogenesis of Hong Kong H5N1 influenza virus NS gene reassortants in mice: the role of cytokines and Band T-cell responses. *J Gen Virol* 2005;86:1121–1130. [PubMed: 15784906]
- Maher JA, DeStefano J. The ferret: an animal model to study influenza. *Lab Anim* 2004;9:50–53.
- Moskophidis D, Kiousis D. Contribution of virus-specific CD8+ cytotoxic T cells to virus clearance or pathologic manifestations of influenza virus infection in a T cell receptor transgenic mouse model. *J Exp Med* 1998;188:223–232. [PubMed: 9670035]
- Pedersen LG, Casteluiz Y, Jacobsen S, Aasted B. Identification of monoclonal antibodies that cross-react with cytokines from different animal species. *Vet Immunol Immunopath* 2002;88:111–122.
- Porter NA, Johnson AH. Rapid speciation via parallel, directional selection on regulatory genetic pathways. *J Theor Biol* 2000;205:527–542. [PubMed: 10931750]
- Robbins PA, McMichael AJ. Immune recognition of HLA molecules downmodulates CD8 expression on cytotoxic T lymphocytes. *J Exp Med* 1991;173:221–230. [PubMed: 1898661]
- Sager H, Davis WC, Dobbelaere DA, Jungi TW. Macrophage-parasite relationship in theileriosis. Reversible phenotypic and functional dedifferentiation of macrophages infected with *Theileria annulata*. *J Lekoc Biol* 1997;61:459–468.
- Saalmuller A, Lunney JK, Daubenberger C, Davis W, Fischer U, Gobel TW, Griebel P, Hollemweguer E, Lasco T, Meister R, Schuberth H-J, Sestak K, Sopp P, Steinbach F, Xiao-Wei W, Aasted B. Summary of the animal homologue section of HLD8. *Cell Immunol* 2005;236:51–58. [PubMed: 16198325]

- Seo SH, Hoffmann E, Webster RG. Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. *Nat Med* 2002;8:950–954. [PubMed: 12195436]
- Singer SH, Noguchi P, Kirschstein RL. Respiratory diseases in cyclophosphamide-treated mice. II. Decreased virulence of PR8 influenza virus. *Infect Immun* 1972;5:957–960. [PubMed: 4635505]
- Thomas PG, Keating R, Hulse-Post DJ, Doherty PC. Cell-mediated protection in influenza infection. *Em Inf Dis* 2006;12:48–54.
- Thompson WW, Shay DK, Weintraub E, Brammer L, Cox N, Anderson LJ, Fukuda K. Mortality associated with influenza and respiratory syncytial virus in the United States. *JAMA* 2003;289:179–186. [PubMed: 12517228]
- Thompson WW, Shay DK, Weintraub E, Brammer L, Bridges CB, Cox N, Fukuda K. Influenza-associated hospitalizations in the United States. *JAMA* 2004;292:1333–1340. [PubMed: 15367555]
- Webby R, Webster RG. Are we ready for pandemic influenza? *Science* 2003;302:1519–1522. [PubMed: 14645836]
- World Health Organization. Cumulative number of confirmed human cases of avian influenza A (H5N1) reported to WHO. Available at http://www.who.int/csr/disease/avian_influenza/en/
- Wright, PF.; Webster, RG. Orthomyxoviruses. In: Knipe, DM.; Howley, PM., editors. *Fields Virology*. Lippincott; Philadelphia: 2001. p. 1533-1579.
- Yan H, Yuan W, Velculescu VE, Vogelstein B, Kinzler KW. Allelic variation in human gene expression. *Science* 2002;297:1143. [PubMed: 12183620]

Figure 1.

Staining profiles for mAbs that showed evidence of cross-reactivity (see Table 1). The PBMCs were from ferrets that had been previously exposed to influenza A virus. A) Histogram and dot plot for staining with the anti-human CD8 clone OKT8. B) Histogram and dot plot staining with anti-mouse CD11b. C-G) Dot plots indicating cross-reactivity with anti-mouse CD44 (C), Thy1.1 (D), CD43 (E), LFA-1 (F), and CD103 (G)

Figure 2.

Surface antigen staining for lymphocytes recovered by BAL of influenza A virus-infected ferrets. The ferrets were first primed with either an H1N1 or H2N3 influenza virus. Several weeks later, they were challenged i.n. with Wuhan H3N2, seven days prior to sampling. A-C) Dot plots of OKT8 (A-C), CD44 (A), CD11b (B), and Thy1.1 (C), which were also cross-reactive on PBMCs in Fig.1. Additional cross-reactivities seen with anti-mouse CD25 (D), CD43 (E), Ly6C (F), and LFA-1 (G) are shown in the subsequent panels. A dot plot representing unstained cells from the ferret BAL is shown in panel H. Results from staining of splenocytes are not shown but are similar to what was seen in cells from the BAL.

Figure 3.

Frequency values for surface antigen positive cells recovered from lung or spleen of influenza virus A-infected ferrets. The percentage of cells staining in BAL (A) and spleen (B) populations is shown for the experiment described in the legend to Figure 2. One ferret that was primed with H1N1 went unchallenged. Spleen cells from a previously-infected mouse were used as previously described (Keating et al., 2007) as a positive control for the mouse mAbs (B).

Figure 4.

Intracellular staining profiles for PMA + ionomycin-stimulated cells from the experiment described in Figure 2. A) Dot plots for staining of cells from ferret BAL with mAbs to bovine IFN- γ and IL-4 that have been previously shown to cross-react with mink. B) Profiles for IFN- γ and TNF in the spleen using anti-mouse antibodies. C-D) Graphs showing the percentage of OKT8+ T cells that were positive for the indicated cytokine in the BAL (C) and spleens (D). Cells from a single mouse spleen were used as a positive control for the mouse mAbs (D).

Table I

Antibodies screened for cross-reactivity with ferret antigens. The table shows the commercially available mAbs that were tested for staining fixed (intracellular) or unfixed (surface) ferret cells. A “low” result indicates minimal staining that could not be considered a true positive result. This summary table is based on findings from all experiments presented in this report.

<i>Antibodies tested against ferret antigens</i>			
Antigen	specificity	clone	result
CD3	mouse	145-2C11	-
CD4	mouse	GK1.5	-
CD4	mouse	RM4-5	low
CD4	canine	YKIX302.9	-
CD8	mouse	53-6.7	low
CD8	human	OKT8	+++
CD11b	mouse	M1/70	+++
CD19	mouse	1D3	low
CD25	mouse	7D4	+
CD27	mouse	LG.3A10	-
CD43	mouse	S7	+
CD44	mouse	IM7	+++
CD62L	mouse	MEL14	-
CD69	mouse	H1.2F3	-
CD103	mouse	M290	+
CD127	mouse	A7R34	-
B220	mouse	RA3-6B2	-
CTLA-4	mouse	1B8	-
Fas	mouse	K10	-
ICAM-1	mouse	3E2	-
LFA-1	mouse	2D7	+
Ly6C	mouse	AL-21	+
Thy1.1	mouse	OX-7	+++
Thy1.2	mouse	53-2.1	-
IL-2	mouse	JES6-5H4	low
IL-3	mouse	MP2-8F8	-
IL-4	mouse	11B11	-
IL-4	bovine	CC303	+
IL-5	mouse	TRFK5	-
IL-10	mouse	JES5-16E3	-
IFN- γ	mouse	XMG1.2	+
IFN- γ	bovine	CC302	+
TNF	mouse	MP6-XT22	+