Ionoclonal Antibodies to Leukocyte ubpopulations in Deer and Exotic :uminants

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'bstract

. significant proportion of monoclonal antibodies developed against specific ovine (54%) and bovine (16.2%) ':ocyte subpopulations cross-react with populations of cells from deer. These monoclonals have been of use to dentify subpopulations of leukocytes within the cervine immune system and to characterize homologous nonoclonal antibodies that have been developed against cervine peripheral blood leukocytes. Antibodies to ine and bovine cells have also been found to cross-react with a variety of exotic ruminants including Père :vid's deer (*Elaphurus davidianus*). Poor levels of cross-reactivity were seen between human or mouse specific nonoclonal antibodies and the ruminants tested in this study. The fact that some leukocyte markers are onserved between ruminants offers researchers the chance to use reagents produced against domesticated ruminants to characterize exotic species.

:ey words: Deer, exotic ruminants, lymphocyte subpopulations, monoclonal antibodies

ntroduction

The use of monoclonal antibodies to examine ubpopulations of leukocytes in the peripheral blood and tissues of animals has allowed rapid 'vances to be made in our understanding of immune responsiveness and disease suscepti- bility. Only recently has this technology been :en out of the laboratory and applied to a broad range of economically or esthetically im- rtant animal species.

The intensive farming of newly domesticated ecies such as deer has given rise to new oblems in disease diagnosis and control (Grif- in 1989). The future economic viability of the deer industry demands an ability to understand, lyze, and ultimately manipulate the immune ponse of animals such that productivity/unit can be maximized. There is also a growing awareness that management of any wild animal in captivity is associated with an increased susceptibility to disease. The immune system of many of these species has been poorly characterized. The production and characterization of mononuclear antibodies to even the basic leukocyte subpopulations of these species will take time and are beyond the scope of most small laboratories. To expedite our ability to analyze the immune response of several exotic species, and red deer (*Cervus elaphus*), in particular, we tested a large number of well-characterized monoclonal antibodies directed against leukocyte subpopulations from better characterized species such as human, mouse, ovine, and bovine in order to determine the usefulness of heterologous antibodies in identifying cervine leukocyte subpopulations.

Materials and Methods

Cross-reactive Monoclonal Antibodies

We studied the following antibodies: ovine specific antibodies that included SBU I(41-17) (Gogolin-Ewens et al. 1985), SBU II(Puri et al. 1985), SBU T19 (MacKay et al. 1986), ST4 (C. MacKay, unpublished data), ST8 (Ezaki et al. 1987) and 197 (M.F. Beya, unpublished data); bovine-specific antibodies that included IL-A24 (Ellis et al. 1988), IL-A29 (Baldwin et al. 1988), 9-9F4 (own unpublished data), CC15 (unpublished data), H4 (Lewin et al. 1985), anti-bovine interleukin-2 receptor monoclonal (Dobbelaere, unpublished data); mouse-specific antibodies-Pgp-1 (Trowbridge 1982); and porcine-specific antibodies-MSA-1 (Hammerberg and Schurig 1986).

Lymphocyte Isolation

Samples of venous blood (9 ml) were drawn into heparinized vacutainers from healthy red deer. Samples were diluted 1:2 with tissue culture medium (RPMI 1640, Gibco), layered onto Ficoll-conray (1.08 g/ml), and centrifuged for 30 min at 400 g. The mononuclear fraction was harvested and washed three time in tissue culture medium. When antibodies specific for macrophage/polymorphonuclear leukocytes were assayed, blood samples were sedimented using 3 ml of 5% Dextran B (150 000–200 000 MW, BDH). Erythrocytes were allowed to sediment for 30 min at 37° C. Residual erythrocytes were lysed for 3 min in 0.85% buffered NH_4Cl before the leukocytes were removed and washed twice in RPMI.

Leukocytes were isolated from lymph node tissue within minutes of animal slaughter and necropsy. Nodes were minced finely with scissors into heparin (10 units/ml) containing RPMI. A single cell suspension was obtained by filtering this material through a fine cotton gauze after which the cells were washed twice, counted, and stained for FACS analysis.

Staining and Analysis of Leukocytes

Using an appropriate dilution (previously determined) of primary antibody $1–2 \times 10^6$ leukocytes were incubated for 20 min on ice. The reaction was terminated by washing three times in PBS, 0.1% BSA, and 0.1% NaN_3 (buffer). Cells were then treated with 1:50 dilution of FITC labeled goat anti-mouse IgG (Sigma, FO257) followed by three further washes in buffer. The last wash included propidium iodide (5 ml/ml of stock solution, 2 mg/ml) to stain dead cells.

A FACSCAN flow cytometer (Becton Dickinson) was used to detect positive cells. Dead cells were gated-out using the red channel (Fl 2), and percent positive cells were defined as those live cells giving readings above those obtained on the same cells using a primary antibody (SBU T1, anti-ovine CD5) shown not to cross-react with deer leukocytes. Ten thousand gated cells were counted in each sample.

Forward vs side scatter was used to determine the purity of cell preparations, and where erythrocyte or platelet contamination occurred these were gated out before statistical analysis.

Leukocytes from the peripheral blood or lymph nodes of at least three animals (except in Fig. 34.2, where cells from only one mature and fetal thymus were obtained, and Table 34.2 where leukocytes from only one ibex, (*Capra ibex*), muscat (*Gazella gazella cora*), and oryx (*Oryx leucoryx*) were tested against each monoclonal antibody to verify cross-reactivity.

In Vitro Cell Culture

Cells isolated as above were resuspended at 2×10^6/ml in RPMI 1640 supplemented with 10% fetal calf serum (Gibco). In 24-well plastic macrotitre plates (Falcon) 1-ml aliquots were cultured for 5 days with 15 mg/ml of concanavalin (Con) A, bovine purified protein derivative (PPD), or avian PPD. Recombinant bovine IL-2 (Immunex) was then added to 10 ng/ml. Every 2 to 3 days, cells were counted, split to 1×10^6/ml, and fed with fresh media containing rIL-2. After 12 days, cells were removed from the wells and stained for FACS analysis.

Results

We assayed 73 leukocyte-specific monoclonal antibodies. Of these, 15 have been shown to cross-react strongly with deer leukocytes. The

TABLE 34.1. Heterologous monoclonal antibodies cross-reactive with cervine leukocyte subpopulations

Monoclonal antibody specificity	Species specificity (% Reactive)		% Cervine PBMs stained positive	
			Mean	SE
Leukocytes				
9.9F4	Bovine	?	94.7	1.6
MHC				
SBU I (class I)	Ovine	>99	99	0.9
SBU II (class II)	Ovine	?	18.9	7.2
B Lymphocytes				
H4	Bovine	33	31	7.9
T Lymphocytes				
ST-4 (CD4)	Ovine	26.6 ± 9.9	24.7	9.2
ST-8 (CD4)	Ovine	15 ± 2.5	17.3	5.8
IL-A29 (T19)	Bovine	7 ± 5.6	15.9	5
CC15 (T19)	Bovine	7.6 ± 5	28	11.2
SBU T19	Ovine	10–30	17.7	6.3
197 (T19)	Ovine	12.8 ± 4.6	17.9	1.6
SBU T6 (CD1)	Ovine	20–30	5.4	1.6
IL-2 Receptor (Tac)	Bovine	?	18.1	5.9
Monocyte/PMN				
IL-A24	Bovine	8.3 ± 2.1	6.8	1.9
MSA-1	Porcine	26.3 ± 3.8	27.5	8.3
Maturation/activation				
Pgp-1 (Ly 24)	Murine (bone marrow)	25–80	87.1	5.9

proportions of cells staining with each of the cross-reactive monoclonals and their target subpopulation in their homologous species are shown in Table 34.1. Of the monoclonals shown to cross-react, none (0/10) were human-specific, and only 1 (1/10) was mouse-specific (Pgp-1). Six bovine-specific antibodies (6/37, 16.2%) were shown to detect cell-surface markers on cervine leukocytes. One of these showed similarities to a ukocyte-common antigen (9-9F4), and IL-A24 etects a subpopulation of monocytes and polymorphonuclear leukocytes (PMNs). The anti-vine B-cell antibody H4 was also cross-eactive and detected a nonimmunoglobulin rker. Its specificity for cervine B cells was etermined by comparing the proportions of H4-sitive and surface immunoglobulin-positive aining cells using anti-cervine monoclonal anti-odies developed in this laboratory (Hibma and F.T. Griffin, unpublished data). A similar pro-rtion of positive lymphocytes was found in sity gradient purified mononuclear cells. H4 pears to be more specific for B cells than anti-immunoglobulin, as in total leukocyte preparations the anti-immunoglobulin monoclonal bound to some myeloid cells (data not shown).

Two bovine-specific monoclonals cross-reacted with a subpopulation of T lymphocytes similar to ovine T19 (MacKay et al. 1986), although CC15 appears to stain less specifically (28% vs 15%–16% for other T19 markers). The T-cell activation marker Tac, a chain of the IL-2 receptor recently found in cattle, also cross-reacted strongly in the cervine system.

Of 13 anti-ovine monoclonals a high proportion (7/13, 54%) was found to cross-react with deer cells (Table 1). Antibodies to the immunologically important CD4, CD8, and major histocompatibility antigens cross-react as did the anti-thymocyte monoclonal T6 (CD1). One of three anti-porcine leukocyte antibodies tested had cross- reactivity with a marker on a subpopulation of cervine monocytes and/or polymorphonuclear leukocytes.

These monoclonal antibodies were used to determine relative proportions of lymphocyte

subpopulations in peripheral blood and lymph nodes of two species of deer, red and Père David's (Fig. 34.1). There was little difference between the two species in the proportions of lymphocytes in each subpopulation, and there was no significant difference between peripheral blood and lymph nodes except in the T19 subpopulation. This population was virtually absent from samples of mesenteric lymph nodes.

When lymphocytes from the thymus of a fetal red deer were compared with those from an adult, significant differences were seen (Fig. 34.2). Once again the T19 subpopulation was absent in both. The fetal thymus contained a large proportion

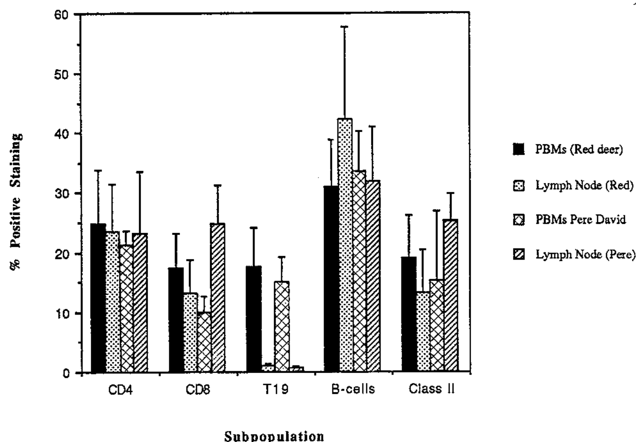

FIGURE 34.1. The relative proportions of B lymphocytes, MHC class II + leukocytes, CD4+, CD8+ and T19+ T lymphocytes found in peripheral blood mononuclear cells (PBMs) and mesenteric lymph-node preparations from red deer ($n = 3$) and Père David's deer ($n = 3$) identified by staining with heterologous monoclonal antibodies and FACS analysis.

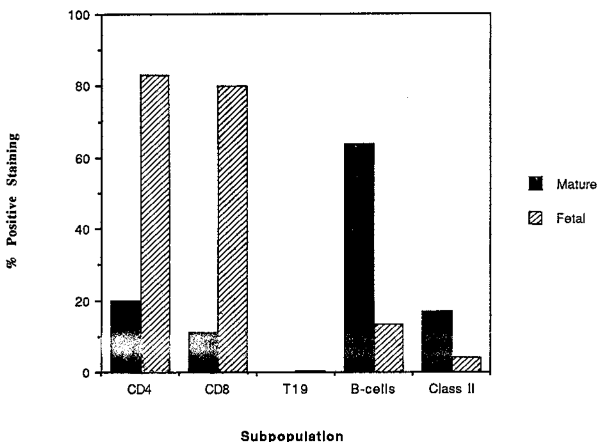

FIGURE 34.2. The relative distribution of lymphocytes among the CD4+, CD8+, B-cell, T19+, and MHC class II subpopulations in the thymus of an adult and fetal red deer using heterologous monoclonal antibodies and FACS analysis.

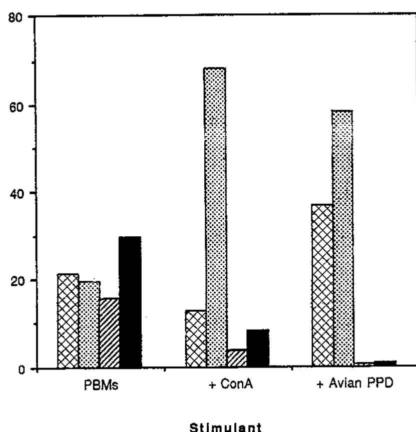

FIGURE 34.3. Changes in the proportions of red deer peripheral blood lymphocytes subpopulations upon in vitro activation with mitogen (concanavalin A) or avian purified protein derivative (PPD). Lymphocytes from an animal with high lymphocyte reactivity to avian PPD were cultured for 5 days with stimulant, then expanded for 9 days with recombinant bovine IL-2.

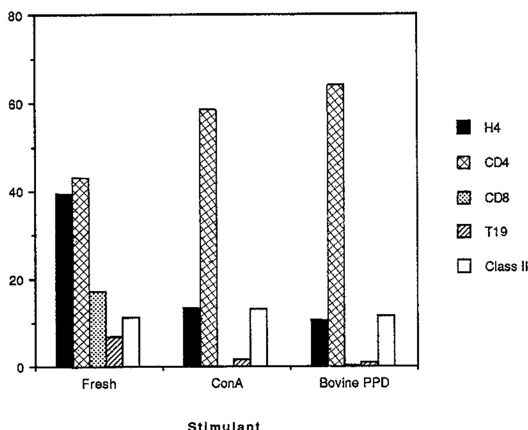

FIGURE 34.4. Proportions of tonsil lymphocyte subpopulations before and after in vitro stimulation with mitogen (concanavalin A) or the specific antigen bovine purified protein derivative (PPD). Lymphocytes from the tonsil of a tuberculosis red deer were incubated for 5 days with stimulant followed by 9 days with recombinant bovine IL-2

~ 80%) of T lymphocytes which were CD4+ ind/or CD8+, few B lymphocytes and few ctivated (class II+) T lymphocytes. The adult ymus had a large proportion of B cells (63.8%) ind relatively normal proportions of CD4 9.7%), CD8 (10.9%), and class II (16.8%) posi-e lymphocytes.

The use of these cross-reactive monoclonals to entify cells which proliferate in vitro to specific gen or mitogen was also investigated. ure 34.3 shows that when grown in culture for 12 days in the presence of rIL-2, the majority of cells exposed to mitogen had the CD8 phenotype, whereas when exposed to specific antigen, avian PPD, both CD4 and CD8 cells proliferated. The numbers of B cells and T19-positive cells decreased with time.

A tonsil preparation from a tuberculous red deer was analyzed for changes in lymphocyte subpopulations during in vitro culture with bovine PPD antigen (Fig. 34.4). The phenotypic compositions of cells from the tonsil were

different from the peripheral blood, and upon culture CD4+ lymphocytes grew out to the exclusion of all other phenotypes.

Discussion

A variety of species possess functionally similar subpopulations of leukocytes which express common surface determinants. The class II reactive (Th) population is identified by the CD4 antigen in man and the L3T4 antigen in mice. A class I reactive population is identified in man by the CD8 antigen and in mice by the Ly2 antigen. Whether antigens such as these are sufficiently conserved to allow for the use of heterologous monoclonal antibodies, raised against one species but used to detect a similar cell type in another species, has not been determined.

While immunological cross-reactivity has been described between major histocompatibility complexes of various species (Lunny et al. 1979; Aasted et al. 1988), the incidence of cross-reactivity in cell phenotyping appears to be much more restricted (Aasted et al. 1988). Our data show that the incidence of cross-reactivity is dependent on the species being studied. None of the human monoclonals used were found to cross-react in deer. Murine- specific antibodies were also found to give a low incidence of cross-reactivity. The solitary success was Pgp-1 which is directed against an antigen that is highly conserved among various species. These data argue against random association between markers on the cells and monoclonal antibodies tested as reasons for the observed cross-reactivity. It appears more likely that the subpopulations detected by cross-reactive antibodies vary between species in the proportion of total leukocytes they contain.

By choosing an appropriate heterologous donor species, in this case ovine or bovine, a much higher incidence of cross-reactivity can be obtained. This may reflect a closer evolutionary relationship between these species or alternatively that ruminants may have evolved similar immune systems to deal with a common antigenic environment. Four of the cross-reactive monoclonals (IL-A29, 197 SBU-T19, and CC15) appear to detect a subpopulation that is similar, if not the same, as that reported in the sheep by MacKay et al. (1986) and seems to be strongly conserved between species.

As pointed out by Aasted et al. (1988) a positive reaction with phenotyping antibodies does not necessarily mean that the cross-reactive monoclonal is binding to the same subpopulation of leukocytes as in the species to which they were developed. Functionally, we have evidence that SBU I and II are seeing similar molecules on cervine and ovine cells (Buchan and Griffin 1990).

The correlation between numbers of lymphocytes staining with H4 and anti-cervine immunoglobulin suggests that H4 does in fact stain cervine B cells. This observation is compelling because it shows that monoclonal antibodies to a surface antigen in one species do identify a functionally similar population, identified by a homologous marker (anti-immunoglobulin) in the host species. Double labeling with anti-cervine immunoglobulin monoclonals will verify this. In addition, the similarities in both reaction patterns and proportions of positive cells between ovine T19, bovine 197, and IL-A29 suggest that the these antibodies also identify a similar marker on cervine leukocytes.

We have begun to use these heterologous monoclonals to probe the immune response of deer, and red deer in particular, in order to better understand the immunological basis of disease problems associated with intensive farm management. Preliminary studies have shown that some subpopulations differ significantly in thier tissue distribution.

Lymphocytes with the T19 phenotype are virtually absent from lymphoid tissues such as mesenteric lymph nodes and thymus, despite being well represented in the peripheral blood and tonsil. These cells are reported to be null cells probably carrying the $\gamma\delta$-antigen receptor. In other species it has been suggested that lymphocytes with these characteristics are localized in specific tissues, usually near epithelial surfaces (Bluestone and Matis 1989).

Although limited to two animals, the phenotypic profiles found in the adult and fetal thymus are intriguing. First it would appear that instead of involuting with age, the cervine thymus develops to take on a role more akin to a lymph node, although a 21-year-old hind was found to

·e very little thymic tissue when autopsied. ıe high proportion of both CD4 + and CD8 + ymocytes in the fetal thymus suggests that ny lymphocytes in the thymus are double itive (CD4 +/CD8 +), immature T lymphotes, as has been reported in other species lokland et al. 1987). In adults this profile has tered to one in which the B-cell predominates hile CD4 + and CD8 + numbers are similar to ·e found in the blood and mesenteric lymph ıode.

The ability to determine the phenotype of the mmunoreactive cells responding to an antigen is basic requirement when studying the immune esponse of an animal. Using heterologous ıonoclonals we have been able to detect differences in the type of lymphocyte that responds) a nonspecific mitogen such as concanavalin A, ɔmpared with a specific antigen such as avain or ɔvine PPD in both peripheral blood and mphoid tissues. This will allow us to identify ıe phenotype of cloned T cells and determine hich cell phenotypes are important in immune rotection to cervine tuberculosis.

Until homologous, leukocyte, subpopulation-cific antibodies become available in newly omesticated species, valuable information on ıe immunology of these species can still be ained by using the huge number of monoclonal ntibodies available as markers of leukocyte ubpopulations in better characterized species. y searching for cross-reactive antibodies and ıen verifying that they identify a functionally milar cell type in the cross-reactive species, we ill be able to more rapidly advance the study of nmunology in the more exotic species of ıminants.

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