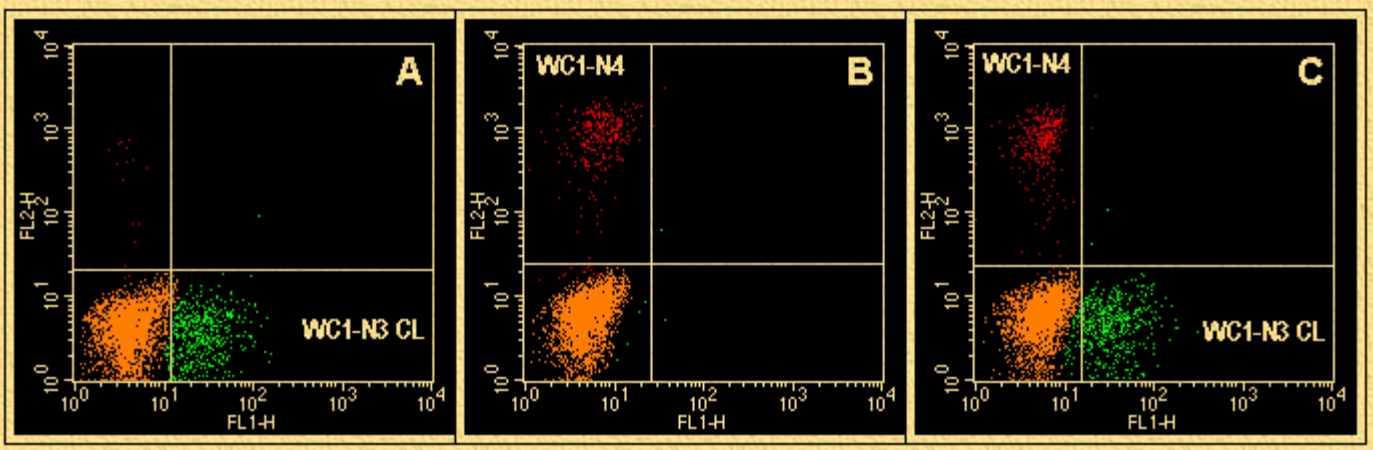

Figure 1: Representative two color dot plot profiles of WC1 positive gamma/delta T cells labeled with monoclonal antibodies that recognize determinants on isoforms of WC1 (WC1-N3 and WC1-N4, [CACT47A and BAQ89A respectively]) that are expressed on mutually exclusive populations of WC1 positive gamma/delta cells. Panel A stained for WC1-N3 alone (X axis). Panel B stained for WC1-N4 alone (Y axis). Panel C stained with both antibodies.

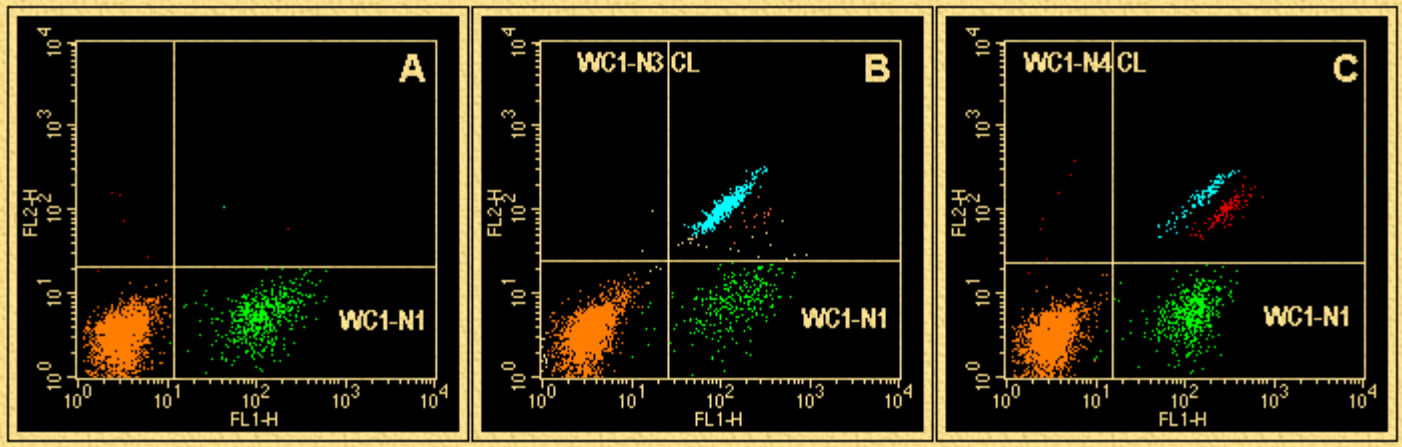

Figure 2: Representative two color dot plot profile of WC1 positive gamma/delta T cells labeled with a monoclonal antibody that recognizes a determinant expressed on the majority of WC1 isoforms (WC1-N1, [B7A]) and monoclonal antibodies that recognize the determinants expressed on mutually exclusive isoforms of WC1 (WC1-N3 and WC1-N4, [CACTB32A and BAQ89A]). The two color pattern of labeling obtained with anti-WC1-N1 and -WC1-N4 a second level of complexity in expression of isoforms of WC1. Panel A stained for WC1-N1 alone (X axis), Panel B stained for WC1-N1 and WC1-N3 (Y axis) and Panel C stained for WC1-N1 and WC1-N4 (Y axis).

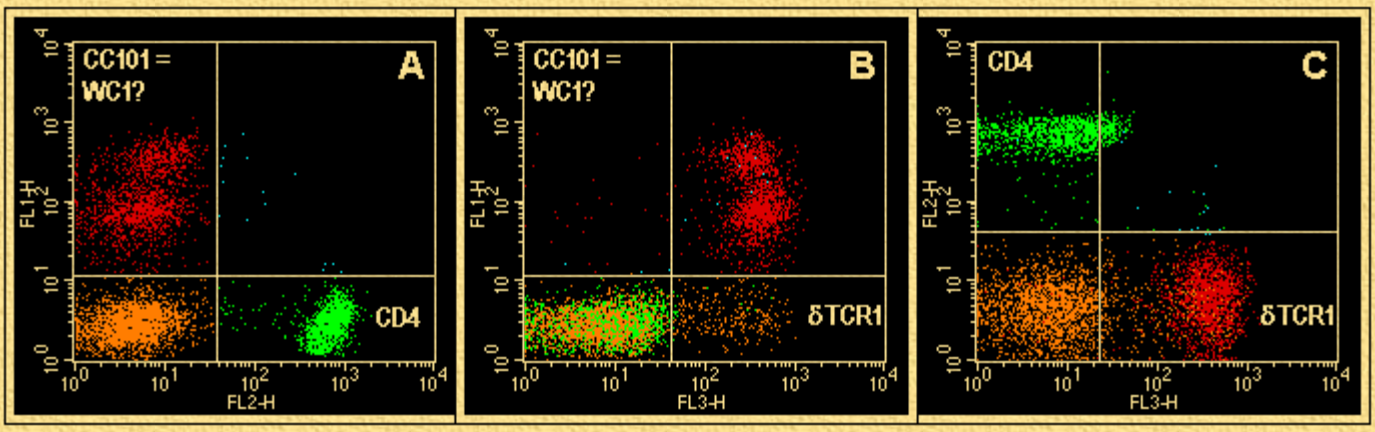

Figure 3: Three color analysis of the pattern of labeling of porcine lymphocytes with monoclonal antibodies that recognize: 1) a conserved determinant on bovine WC1 molecules and the putative orthologue of WC1 in swine (CC101), 2) a determinant on the delta chain of the gamma/delta TCR (PGBL22A), and 3) a determinant expressed on CD4 (74-12-4). As noted in panel A (Y axis) CC101 recognizes two populations of cells that express different levels of the CC101-defined molecule. These populations of cells co-express the gamma/delta TCR (panel B, Y and and X axes). Panels A and C show CD4 is not expressed on gamma/delta positive cells.

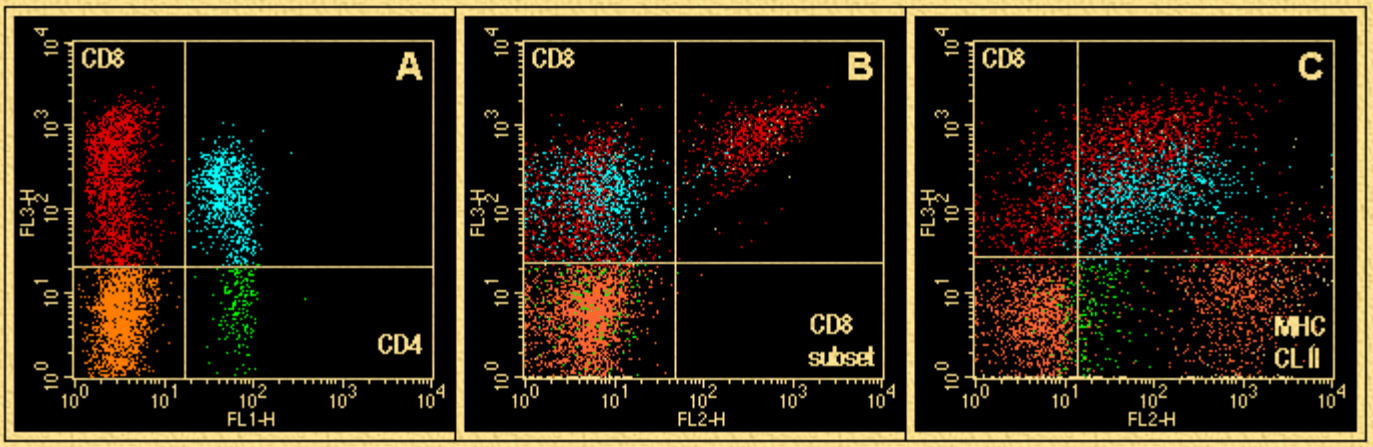

Figure 4: Three color analysis of the pattern of expression of CD4 (X axis), CD8 (Y axis), and MHC class II molecules (X axis) on porcine lymphocytes. Panel A shows a large subset of CD4 positive cells co-express CD8. Panel C shows these cells co-express MHC class II. Panel B shows the bright staining population of CD8 positive cells that co-express CD8 alpha and putative CD8 beta are negative for CD4. Panel C shows this population of cells co-express MHC class II.

G00061

Unique Characteristics of the Immune Systems in Ruminants and Pigs

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Abstract:

Comparative analysis of the immune systems of food and companion animals has revealed differences in the composition of T lymphocyte subpopulations in ruminants and pigs. The difference in ruminants is attributable to the presence of a major subset of gamma/delta T cells that appears to have undergone expansion in the course of evolutionof ruminants. This subset is characterized by the expression of two lineage restricted molecules, WC1 (bovine workshop cluster 1) andGD3.5 and restricted usage of gamma genes of TCR1 and Vgammasequences. The difference in pigs is attributable to the presenceof a similar orthologous subset of gamma/delta T cells and the expression ofCD4, CD8, and MHC class II molecules on alpha/beta T cell subsets. As inruminants, the unique gamma/delta T cell subset is characterizedby the expression of two lineage restricted molecules, one thatappears to be the porcine orthologue of WC1 and WC6 (porcineworkshop cluster 6). No information is available on the possiblerelation of GD3.5 and WC6. In contrast to other species, alpha/betaT cells are divided into multiple subsets characterized by thedifferential expression of CD4, CD8alpha/alpha, CD8alpha/beta, andMHC class II molecules. These observations suggest the mechanismsregulating the development and expression of immune responses toinfectious agents and derived vaccines may differ from thosedescribed in rodents and humans.

Introduction:

Ongoing comparative studies have revealed the immune system in many species of mammals is similar to the immune systems noted in humans and rodents. The major populations of leukocytes can be distinguished by flow cytometry based on forward and side light scattering properties and the expression of lineage and non-lineage restricted leukocyte differentiation molecules. The leukocyte differentiation molecules that have been identified thus far have proven to be highly conserved in structure and function. The pattern of expression of the molecules has also been conserved. This has proven useful in identifying and characterizing monoclonal antibodies (MoAbs) that recognize determinants on orthologous molecules using flow cytometry(1-3). Two important exceptions have been noted, however. The first has been in the expression of the major histocompatibility class II molecules on resting T lymphocytes in some species. In contrast to expression in rodents and humans, MHC class II molecules are expressed on resting T lymphocytes in horses, dogs, cats, and swine (2). The second and most striking exception has been in the composition of the immune system in Artiodactyla (4-7). Examination of the immune system in ruminants and swine has revealed a difference in the composition of the gamma/delta T cell population in both groups of animals and in addition, a difference in the composition of alpha/beta T cells in swine (8). The objectives of this presentation are to summarize recent observations that indicate a subset of gamma/delta T cells has undergone expansion in the course of evolution of Artiodactyla and observations that show unique differences in the composition of alpha/beta T cell populations in swine.

Materials and Methods:

Blood was collected from animals maintained according to AALAC and the Animal Use and Care Committee guidelines at Washington State University or in the case of swine, at the time of slaughter. Leukocytes were prepared for two and three color flow cytometric analysis as previously described (9). Becton Dickinson FACSort and FACScan flow cytometers equipped with Macintosh computers and Cell Quest software were used to collect and analyze data. The following MoAbs were used to prepare the figures for this presentation: B7A (IgM, recognizes the WC1-N1 determinant), CACTB32A and CACT47A (IgG1 and IgM respectively. Both recognize determinants on the WC1-N3

isoform), BAQ89A (IgG1, recognizes a determinant on the WC1-N4 isoform), CC101 (IgG2a, recognizes a conserved determinant on WC1 in cattle and swine), PGBL21A (IgG1, recognizes a determinant on the delta chain of the gamma/delta T cell receptor [TDR1]), 74-12-4 (IgG2b, recognizes CD4). CC101 was a gift from C. Howard, AFRC, Compton, UK. 74-12-4 was obtained from VMRD, Inc., Pullman, WA.

Results:

The difference in the composition of the gamma/delta T cell population in ruminants and swine is attributable to the presence of an unique subset of gamma/delta T cells. In cattle, the subset is characterized by the expression of two glycosylated high molecular weight lineage restricted molecules, workshop cluster 1 (WC1) (10) and GD3.5 (11). Analysis of WC1 has shown it is a member of the recently described scavenger receptor cysteine-rich (SRCR) family of molecules that includes CD5 and CD6 (12-14). The gene encoding the molecule is present in multiple copies (13). It is not yet clear how many copies can be expressed at the same time. However, as illustrated in figure 1, there are at least two MoAb-defined isoforms that are present on mutually exclusive populations of WC1 positive gamma/delta cells, WC1-N3 and WC1-N4 (15). Previous studies have shown the two WC1 genes that have been cloned and expressed in L cells WC1.1 and WC1.2 encode the WC1-N4 and WC1-N3 isoforms respectively (15). As illustrated in figure 2, these populations are included in the WC1 positive population defined with a MoAb that recognizes a determinant present on both the WC1-N3 and WC1-N4 isoforms. The diagonal pattern of labeling indicates there is an additional level of complexity in expression of WC1 isoforms. The double diagonal array noted with WC1-N4 suggests that multiple isoforms may be expressed on the same subset of gamma/delta cells or alternatively, different levels of expression of an isoform on the same subset. More complex patterns of labeling have been obtained with other combinations of MoAbs that support the contention that multiple isoforms may be expressed on the same or overlapping subsets of WC1 positive gamma/delta cells (data not shown).

Less is known about the GD3.5-defined molecule (11). However, the data that have been obtained on its pattern of expression suggest there is only one copy of the molecule and that it is expressed on all WC1 positive cells.

As in cattle, the unique subset of gamma/delta T cells in swine is characterized by the expression of two lineage restricted glycosylated high molecular weight molecules (6,12). As illustrated in figure 3, two color analysis with a MoAb that recognizes a conserved determinant expressed on bovine WC1 and the apparent orthologue of WC1 in the pig and a MoAb specific for the delta chain of porcine TCR1 show the population identified with CC101 is included in the population recognized by the anti-delta chain MoAb, PGBL21A. Consistent with observations in cattle with anti-WC1 anitbodies, the population positive for CC101 does not express CD2 or CD8 (data not shown). CC101 is the only characterized MoAb that has been found to react with the putative porcine orthologue of WC1. Consequently, at this juncture it is not known whether multiple isoforms of this molecule exist.

The second molecule, referred to as swine workshop cluster 6 (SWC6), has a pattern of expression identical to CC101 ((6), personal observations). It is not expressed on CD2 positive gamma/delta cells. Two color analyses with leukocytes from some animals suggest the original MoAb used to define this molecule (MAC320), labels more gamma/delta T cells than CC101, suggesting the determinant recognized by CC101 is not expressed on all porcine WC1 equivalent molecules. Currently, there is no information on the possible relation of GD3.5 and SWC6.

Analysis of the alpha/beta T cell population in swine has revealed it is more complex than the alpha/beta T cell population noted in other species (8). As illustrated in figure 4, 3 color analysis has shown the alpha/beta T cell population is comprised of multiple subsets characterized by the differential expression of CD4, CD8alpha/alpha homodimer, CD8alpha/beta heterodimer, and MHC class II molecules. Labeling with anti-CD8alpha yields a broad pattern of staining intensity from very bright to dim (see panel A). Co-labeling with anti-CD4 MoAb shows that a subset of dim staining CD8alpha/alpha positive cells co-express CD4. Inclusion of a MoAb that recognizes CD8beta as the third antibody shows the majority of bright staining CD8 positive cells express the alpha/beta heterodimer (panel B). Inclusion of a MoAb specific for MHC class II shows that the bright staining CD8 heterodimer and the dim staining CD4 positive CD8 homodimer populations co-express MHC class II (panel C). In addition to these differences the 3 color staining shows some CD4 positive cells and a subset of dim staining CD8 homodimers do not express CD4 or MHC class II.

Discussion and Conclusions:

Although additional studies are needed, the cumulative data suggest the evolution of T cells in Artiodactyla differs from that noted in other species. The studies in cattle, as a representative ruminant, and swine indicate the unique subset of gamma/delta T cells may have a common phylogenetic origin. This is based on the finding of two lineage restricted molecules expressed on the subset in both species, evidence that one of the molecules appears to be the orthologue of WC1, the pattern of expression of the second molecules, GD3.5 and SWC6, and tissue distribution. In each species, the gamma/delta population is divided into two major subpopulations, one subpopulatio positive for WC1 or the equivalent and GD3.5 or SWC6 and negative for CD2, CD6, and CD8 and the other subpopulation, positive for CD2 with a subset that co-expresses CD8 and negative for WC1 or the porcine equivalent and negative for GD3.5 or SWC6. The bovine and porcine WC1 equivalent populations of gamma/delta T cells are present in high concentration in peripheral blood of young animals and low in secondary lymphoid organs ((16), and personal observations). The CD2 and CD2,CD8 positive equivalent populations are present in low concentration peripheral blood and high in spleen, and mammary tissue. This population has also been found in high concentration in gut epithelium in cattle (personal observations). Equivalent studies have not yet been performed in swine. Cloning and sequencing of the gene encoding the putative WC1 molecule in swine and the genes encoding GD3.5 and SWC6 will show whether the apparent similarities in the composition of the unique population of gamma/delta cells reflects a common origin.

The difference in the composition of the alpha/beta T cell population in swine appears to be unique to the pig. Comparative studies have not revealed any other species with a similar composition.

The differences noted in the composition of the immune systems in ruminants and swine suggest the mechanisms regulating the development and expression of immune responses to infectious agents and derived vaccines may differ from those described in humans and rodents. It is now evident that an additional unique population of gamma/delta cells is present in both groups of animals and possibly in all Artiodactyla. The distribution of this population in blood and tissue differs from the CD2 positive population indicating the two populations of gamma/delta cells may play different roles in regulation and expression of immune responses. Further studies are now needed that focus on elucidating how these two subpopulations gamma/delta T cells interact and function during immune responses to infectious agents.

Additional studies are also needed to elucidate the functional significance of the differences in the composition of the alpha/beta T cell population in swine. It is clear that subsets of resting (unstimulated) CD8 positive and CD8/CD4 positive alpha/beta T cells express MHC class II molecules. This suggests these populations of cells could behave as antigen presenting cells and be involved in regulation of immune responses through the production of unique sets of cytokines. The role of CD8/CD4 positive T cells in the development and expression of immune responses remains unclear, but recent studies suggest this population of cells is comprised in part, of memory T cells. The finding of a population of CD8 alpha/beta T cells that is MHC class II positive is very recent and there has been little opportunity to examine the role of this population in development, regulation, and expression of immune responses.

The observations presented here should provide opportunity for dialogue and discussion of ongoing studies in other laboratories.

References:

1. Lanier, L. L., E. G. Engleman, P. Gatenby, G. F. Babcock, N. L. Warner, and L. A. Herzenberg. 1983. Correlation of functional properties of human lymphoid cell subsets in surface marker phenotypes using multiparameter analysis and flow cytometry. Immunol. Reviews 74:143.

2. Davis, W. C., S. Marusic, H. A. Lewin, G. A. Splitter, L. E. Perryman, T. C. McGuire, and J. R. Gorham. 1987. The development and analysis of species specific and cross reactive monoclonal antibodies to leukocyte differentiation antigens and antigens of the major histocompatibility complex for use in the study of the immune system in cattle and other species. Vet. Immunol. Immunopathol. 15:337.

3. Davis, W. C., R. A. Larsen, and M. L. Monaghan. 1990. Genetic markers identified by immunogenetic methods. Am. Fisheries Soc. Symp. 7:521.

4. Binns, R. M., I. A. Duncan, S. J. Powis, A. Hutchings, and G. W. Butcher. 1992. Subsets of null and td T-cell receptor + T lymphocytes in the blood of young pigs identified by specific monoclonal antibodies. Immunology 77:219.

5. Hein, W. R. and C. R. Mackay. 1991. Prominence of td T cells in the ruminant immune system. Immunol. Today 12:30.

6. Licence, S. T., W. C. Davis, M. M. Carr, and R. M. Binns. 1995. The behaviour of monoclonal antibodies in the First International Pig CD Workshop reacting with td/ Null T lymphocytes in the blood of SLAb/b line pigs. Vet. Immunol. Immunopathol. 47:253.

7. Ishiguro, N. and W. R. Hein. 1994. The T cell receptor. In Cell-mediated immunity in ruminants. B.M.L. Goddeeris and W.I. Morrison, eds. CRC Press, Boca Raton, p. 59.

8. Pescovitz, M. D., A. G. Sakopoulos, J. A. Gaddy, R. J. Husmann, and F. A. Zuckermann. 1994. Porcine peripheral blood CD4+/CD8+ dual expressing T-cells. Veterinary Immunology and Immopathology 43:53.

9. Davis, W. C., J. E. Davis, and M. J. Hamilton. 1995. Use of monoclonal antibodies and flow cytometry to cluster and analyze leukocyte differentiation molecules. In Monoclonal Antibody Protocols, Methods in Molecular Biology, Vol. 45. W.C. Davis, ed. The Humana Press Inc, Totowa,NJ, p. 149.

10. Morrison, W. I. and W. C. Davis. 1991. Differentiation antigens expressed predominantly on CD4-CD8- T lymphocytes (WC1,WC2). Vet. Immunol. Immunopathol. 27:71.

11. Jones, W. M., B. Walcheck, and M. A. Jutila. 1996. Generation of a new td T cell-specific monoclonal antibody (GD3.5): biochemical comparisons of GD3.5 antigen with the previously described workshop cluster 1 (WC1) family. J. Immunol. 156:3772.

12. Carr, M. M., C. J. Howard, P. Sopp, J. M. Mauser, and K. R. Parsons. 1994. Expression on porcine td lymphocytes of a phylogenetically conserved surface antigen previously restricted in expression to ruminant td T lymphocytes. Immunology 81:36.

13. Wijngaard, P. L. J., N. D. MacHugh, M. J. Metzelaar, S. Romberg, A. Bensaid, L. Pepin, W. C. Davis, and H. C. Clevers. 1994. Members of the novel WC1 gene family are differentially expressed on subsets of bovine CD4-CD8- td T-lymphocytes. J. Immunol. 152:3476.

14. Resnick, D., A. Pearson, and M. Krieger. 1994. The SRCR superfamily: a family reminiscent of the Ig superfamily. Trends Biochem. Sci. 19:5.

15. MacHugh, N. D., P. L. J. Wijngaard, H. C. Clevers, and W. C. Davis. 1993. Clustering of monoclonal antibodies recognizing different members of the WC1 gene family. Veterinary Immunology and Immopathology 39:155.

16. Wyatt, C. R., C. Madruga, C. Cluff, S. Parish, M. J. Hamilton, W. Goff, and W. C. Davis. 1994. Differential distribution of td T cell receptor positive lymphocyte subpopulations in blood and spleen of young and adult cattle. Veterinary Immunology and Immopathology 40:187.

Comments:

Address questions and comments about this abstract to William (Bill) C. Davis (davisw@vetmed.wsu.edu).

Questions and Answers

Q: I looked at your poster in the NADC virtual conference and found that you mention one of your antibodies PGBL21a recognizing the delta chain of the gd TCR in materials and methods. On the VMRD-webpage, however, I found this Mab is called PGBL22a. Which one is real? I know you also tested other MAb's with similar designations for the second swine CD workshop. The workshop report from Immunology Today mentions PGBL23A among the CD2a cluster. Is this correct or also a typo? In which cluster did PGBL22A "land"?

A:You are right on PGBL22A. I was thinking of a MoAb GB21A that recognizes the delta chain in goats and cattle. It was a struggle to get the poster in before time ran out. The data indicate PGBL22A recognizes the delta chain in swine. It does not cross react with TCR1 in other species. Because it is specific for TCR1 delta chain, it did not cluster per se. It did group with the other MoAbs I developed that appear to detect determinants on the gamma chain. 86D groups with these MoAbs as a subset of TCR1 positive lymphocytes. The summary report that will come out late this year will have more details. PGBL23A is correct. It is isotype IgG1. This MoAb hasn't been licensed since MSA4 (IgG2a) and PG168A (IgG3) are available for general use. The MoAb is available through the WSU MoAb Center if needed by any investigator.

Q:Which Mab was used for panel B in figure 4 for labeling the CD8 (beta) subset (Fl2)?;

A: I used PG164A (IgG2a).

Q:In figure 3 gamma/delta single positive cells appear. Did you test whether these are actally positive for MAC320? In the results you speculate on the comparison between CC101 and MAC320.

A: I used MAC320 with CC101 and also PGBL22A in two and three color analyses. Again, the report in the summary of the last workshop has profiles of the different definitive combinations. To appreciate what you will read in the report, it would be worthwhile to read the report in the 3rd workshop on ruminant leukocyte differentiation molecules: VII 52:275, 1996. The results in swine are very similar to those in ruminants. As mentioned the unique subset of gamma/delta cells expresses the WC1 molecule and an additional molecule. This population in swine is positive for MAC320. The MAC320/CC101 negative population is positive for CD2. The concentration of this population is high in the spleen and low in peripheral blood with the opposite being true for the MAC320 positive population. MAC320 has been finally licensed to SEROTEC so it is available to everyone for studies. I tried to contact Tony Whyte to see if they are attempting to clone and express MAC320 to see what the molecule is. My address is no longer functional so I didn't get an answer.

Q:Another question concerning your Mab PGBL22A. We and others encountered problems in double labeling experiments with Mab of the isotype IgG2a. After testing, we found this antibody has a heterogeneous isotype composition. This, however, is not apparent from figure 3 of your poster G00061 at the NADC virtual conference, where CC101 (Fl1) is IgG2a and PGBL22A is on the Fl3 axis. Have you cleaned up the Mab in the mean time or recloned it?

A:It would appear there are some technical problems with labeling. I am using aliquots of the same lot as provided through VMRD. You should, however, provide me with the lot date so that I can be sure nothing has gone awry. I have had background staining problems every so often that proved to be attributable to the proper adherence or nonadherence to the staining protocol. You need to describe your staining procedures to see if I can ascertain what might be happening in your studies.

Q:What immunological challenges unique to Artiodactyla might be responsible for the evolutionary pressure to develop this unique subset of gamma/delta T cells?

A:As with gamma/delta T cells cross species, data remain limited on the role of gamma/delta T cells in regulation and

expression of immune responses to infectious agents and parasites. At this juncture, studies indicate gamma/delta cells play an important role in first line defense. Response is not MHC restricted. TCR1 is similar to antibody in that TCR1 recognizes determinants present on unprocessed antigen. Epitopes recognized by TCR1 vary in molecular composition and are not restricted to peptides. What appears to be emerging from studies of the unique population in ruminants and swine (representative Artiodactyla?) is that there is restricted usage of V gamma segments in the TCR. In addition it appears the population is more mobile than the WC1 negative population with no particular site of lodging. The WC1 positive population appears to be trafficking through all tissues with a short transit time unless the cells encounter signals that arrest migration at sites of infection (or injury?). Studies are needed to elucidate the function of the WC1 molecule in trafficking and costimulation following engagement of TCR1 with antigen. Studies are also needed to elucidate the role of the additional unique molecule identified in each species.

Q:What causes the gradual decline in the percentage of circulating WC1+ cells as an animal ages? And do you see a concomitant increase in WC1+ cells in secondary lymphoid organs?

A: One can only speculate as to the reasons for decline in the percentage of WC1+ cells in circulation with age. Their prevalence in young animals suggest these cells play an important role in defense in young animals. There is no evidence at this juncture, that the decline in concentration is associated with lodging in any tissue in aging animals. What is needed to answer the question is information on the specificity and function of both WC1+ and WC1- gamma/delta T cells.