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Use of flow cytometry to characterize immunodeficiency syndromes in camelids☆

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Abstract

Disorders in immune function in lamas have been observed over the past years. However, it has been difficult to determine how many types of deficiencies exist. Through the use of flow cytometry and monoclonal antibodies specific for leukocyte differentiation molecules, it has been possible to characterize the immune system of lamas and determine the genetic basis of one disease, the juvenile lama immunodeficiency syndrome (JLIDS). The availability of monoclonal antibodies and flow cytometry now afford an opportunity to clinically diagnose animals with JLIDS at birth and characterize other immunodeficiencies in animals presenting with similar clinical signs of immune dysfunction. The findings also show that flow cytometry can be used to characterize disorders in other species.

Keywords: Llamas; Alpacas; Immunodeficiency; Flow cytometry

1. Introduction

Neonatal diseases with high morbidity and mortality have remained a serious problem in the lama industry in South America and well as in North America. Although it has been suspected for some time that ill thrift and recurrent infections in some affected crias may be, in part, hereditary and associated with dysfunction of the immune system, diagnosis has been difficult owing to the lack of knowledge on the composition and function of the immune system in camelids and also the lack of methods to detect defects in immune function. Studies in humans suggest multiple types of defects might also occur in camelids and account for observed health problems. Studies have revealed mutations in genes that interfere with the development and function of the immune system. Over 100 genetic disorders affecting immune function have been characterized in humans since 1952, when the first inherited immunodeficiency disorder was described (see review (Buckley, 2002). These include X-linked and autosomal defects in genes regulating the development and maturation of lymphocytes involved in cellular (T lymphocytes) and humoral immunity (B lymphocytes) or selective

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defects in expression and/or secretion of gene products involved in regulation and expression of immune responses. Other mutations have affected the capacity of phagocytic cells to take up and kill pathogens (Davis et al., 1968b,a; Davis and Douglas, 1972; Dinauer et al., 1987; Douglas et al., 1969). Some of the same genetic mutations have also been identified in horses (Felsburg et al., 1992; Perryman, 2000), cattle (Kehrli et al., 1992; Rutten et al., 1996), and dogs (Entman et al., 1990; Giger et al., 1987; Renshaw and Davis, 1979). The success in identifying and characterizing these genetic disorders is attributable to a series of technological advances that have been made over the past 40 years. Two particular advances that facilitated research on the immune system and the characterization of immunodeficiency disorders in humans were the development of monoclonal antibody (mAb) technology (Kohler and Milstein, 1975) and flow cytometry (Herzenberg et al., 2000). Monoclonal antibodies have provided the tools needed for identification and functional analysis of cells involved in regulation and expression of immune responses. Flow cytometry in conjunction with mAbs has afforded a way to study the interaction of lymphocyte subsets during the development and expression of immune responses. Over 247 mAb-defined molecules expressed on all or subsets of leukocytes have been identified in humans and assigned a numerical designation, cluster of differentiation (CD1, CD2, etc.) (Mason et al., 2002). The advances made in characterization of the immune system in humans and laboratory animals have provided information that has greatly facilitated similar studies in food and companion animals. Two observations to emerge from comparative studies that have aided in the analysis of the immune system in ruminants, pigs, and lamas, members of the order Artiodactyla (suborders Ruminantia, Suiformes and Tylopoda, respectively) are one, that the molecules expressed on leukocyte populations (leukocyte differentiation molecules) are highly conserved in structure and function in different species of mammals. The finding that some of the mAbs recognize determinants conserved on equivalent (orthologous) molecules in other species allowed for the direct identification of orthologous molecules in species under study (Davis et al., 1987; Davis and Ellis, 1991; Muriuki et al., 1998; Naessens et al., 1993). The second observation is that the pattern of expression of most of the leukocyte differentiation molecules is also highly conserved and that flow cytometry can actually be used to identify and cluster mAbs predicted to recognize orthologous molecules (Davis et al., 1987, 1995; Lanier et al., 1983, 1986). Both observations have proven useful in the identification of mAbs for use in the characterization of immune systems in less well-studied species, especially in camelids where resources have been extremely limited. The CD nomenclature used to tabulate molecules defined by clusters of mAbs in humans has been adopted for use in other species with a prefix to minimize confusion when discussing the properties of orthologous molecules in different species (e.g. bovine CD2 [boCD2, boCD3, boCD4, boCD8, etc.]) (Naessens et al., 1997; Saalmüller, 1996). Where identity of mAb-defined molecules has not been established, a temporary workshop cluster designation has been used to define the cluster (e.g. WC1, WC2, etc.). Progress in developing and characterizing mAbs for use in ruminant and pig research are summarized in the last international workshops (Haverson et al., 2001; Naessens and Hopkins, 1996). Information on mAbs characterized for use in camelids has been summarized by Hamers and Muyldermans (1998) and Davis et al. (2000).

2. Immune system of camelids

One important observation to emerge from comparative studies of the immune system in mammals is that the immune systems in different species are similar but not identical. Differences in the composition and frequency of lymphocyte subpopulations exist that need to be taken into consideration when attempting to analyze the immune response to pathogens and vaccines. Studies of the immune system in Artiodactyla have revealed unique differences not found in other orders of mammals. Differences have been noted in the composition of αβ and γδ T lymphocytes in the pig, γδ T lymphocytes in ruminants and camelids, and B lymphocytes in camelids. In species that have been examined thus far, $CD4^+$ (T helper) and $CD8^+$ (T cytotoxic) αβ T lymphocytes are mutually exclusive subpopulations, each comprised of naïve and memory T lymphocytes. In vitro stimulation with recall antigens in immunized animals elicits an antigen specific proliferative memory T lymphocyte response in either or both $CD4^+$ and $CD8^+$ populations. Double positive lymphocytes only