A Review of Observations Made on Select Parameters of the Camel Immune System

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ABSTRACT: Despite the camel’s long historical interaction with man there is only a limited number of studies available pertaining to the immunobiology of this species. This is unfortunate as the camel has evolved into an animal capable of not only surviving under extreme environmental conditions but also one that is relatively resistant to a great number of infectious diseases. Accordingly, it is of interest to understand the various components operative in the camel immune system, as a potential basis of manipulating the immune response of other domesticated animals to respond to disease-causing agents in a similarly effective fashion. Recent research endeavors on the complement and phagocytic system, as well as the unique antibody types found in camelids that have seen an explosion of interest in recent times have been reviewed and their potential use as diagnostic and therapeutic tools highlighted.

Keywords: Camel, immune system, antibodies, leukocytes.
to destroy invading organisms, and equally important, to act as a signaling system for other elements of the immune system. In order to keep the complement system in check the individual complement components must be activated before they become effector molecules. This can be achieved by one of three ways. The first way is mediated through antibodies and is termed the ‘classical pathway of complement fixation’. The second way is termed the alternative pathway. Finally, the complement system can be activated in a more recently-described fashion termed the lectin activation pathway (Weiss et al., 1992). The result of all three pathways is the generation of a series of effector molecules. A generated membrane attack complex (C5b-C9) causes lysis of foreign organisms. The complement system also produces proteins that serve as opsonins (iC3b, C3b, C4b), and chemoattractants (C3a, C4a and C5a) that induce acute inflammation by binding to mast cells and triggering their degranulation and subsequent release of vasoactive mediators, such as histamine. In addition, these peptides can stimulate chemokines of neutrophils, adherence to endothelial cells and enhance the respiratory burst of phagocytic cells.

Camels appear to have demonstrable levels of classical pathway hemolytic activity throughout their lives (Olaho-Mukani et al., 1995a) and similar to lambs (Oswald et al., 1990) and calves (Jain and Goel, 1989) appear to acquire relatively large amounts of hemolytic complement activity via colostrum. They only exhibit higher levels of classical complement activity in their sera from 1-5 years of age (Olaho-Mukani et al., 1995a). Similarly, it has been reported that camels between 3 months and one year of age have higher levels of alternative complement pathway activity in their sera than camels in the age group of five years and above (Olaho-Mukani et al., 1995b). It is unknown why the complement activity declines but it is unlikely due to the presence of conglutinins in serum, as described in cows (Kakoma and Kinyanui, 1974), as only low levels of these proteins have been found in the sera of camels (Bhatnagar et al., 1987). Interestingly, older male camels have been reported to exhibit higher levels of classical hemolytic complement activity than females of the same age group (Olah-Mukani et al., 1995a). Although one can hypothesize that this is merely a hormone related phenomenon it does not readily explain what biological function this might serve. It would be interesting to determine whether older female camels are in any way more prone to infectious agents than similar aged male counterparts. Besides for the defense against microorganisms, complement appears to be important in the control of parasitism in camels infected with Trypanosoma evansi. Also, it has been suggested that the decreased levels of complement activity commonly observed in animal trypanosomiasis might result in immunosuppression (Ouma et al., 1997).

The Neutrophil

Polymorphonuclear neutrophils play a crucial role in the phagocytosis and killing of invading microorganisms. They are also the predominant leukocyte type found in the peripheral blood of camels (Abdurahman et al., 1992; Wernery, 1995). In camels their numbers vary with age. Young camels tend to have larger numbers of white blood cells and a higher percentage of neutrophils than lymphocytes (Wernery et al., 1999). This is in contrast to most ruminants that have a neutrophil:lymphocyte ratio approximating 1:2. The neutrophils of camels are reported to be smaller than those from other animal species (Ali et al., 1989). A unique ultra-structural observation in the camel, not found in other animal species, is the finding that the nucleus is bound by a well-defined perinuclear space (Ali et al., 1989). Camel neutrophils appear to have highly electron dense and less dense granules that likely correspond to the primary and specific granules reported in most other animal species (Ali et al., 1989). Tertiary granules have not been demonstrated. Camel neutrophils exhibit an oxidative respiratory burst similar in its kinetics to that of neutrophils of other species against Staphylococcus aureus (Cooray et al., 1997) However, our lab has observed that neutrophils from camels exhibit a higher level of activity in their chemiluminescence response than sheep (Johnson et al., 2006).

The Eosinophil

Eosinophils are most commonly associated with the ability to kill multicellular parasites and for their role in specific types of allergic reactions. However, eosinophils play an important role in regulating inflammation by modulating the function of mast cells, basophils and eosinophils (Gleich and Adolphson, 1986). They also produce inflammatory mediators such as leukotriene C4 (Jorg et al., 1982; Shaw et al., 1984), platelet activating factor (Lee et al., 1984), oxygen free radicals (Tauber et al., 1976) and cationic proteins (Olson et al., 1986; Peterson et al., 1986; Peterson and Venge, 1983). There is evidence that eosinophils might express major histocompatibility antigens after stimulation by granulocyte-macrophage colony stimulating factor (Weller, 1992). Accordingly, they might play a role in antigen presentation. Neither a structural nor functional biological equivalent to IgE has been found in the camel, so it is unknown whether the camel eosinophil has an Fc receptor for this or any of the three camel IgG subclasses. At the ultra-structural level the camel eosinophil has unique characteristics. Their specific granules exhibit the basic structure of an electron-dense crystalloid core surrounded by a lighter, homogenous matrix. However, in contrast to other animal species, they are very polymorphic. The crystalloid cores are extremely variable in shape and size and often segmented and demonstrate a variety of lamellated patterns. Interestingly, it is not unusual to demonstrate multiple crystalloid cores in a single granule. The extreme polymorphism of the specific granules and variety of lamellated patterns differentiate camel eosinophils from those of other species (Johnson et al., 1999). Camel eosinophils also appear to be especially effective killers.
of parasites and when activated commonly reveal hyper-
segmented nuclei (unpublished observation). It is not yet
clear how these structural differences render the camel
with such an apparent efficiency in killing parasites.

Camelid Antibodies

Antibodies are generally described as protein molecules
consisting of two pairs of proteins, termed heavy chains
and light chains. The prototype antibody consists of an
antigen binding region (Fab), made from both heavy and
light chain segments, and an Fc fraction that is made
entirely from the heavy chain. Antibodies are generally
considered to be divalent and bi-functional. Divalency
refers to their ability to bind two antigens at the same
time. The bi-functionality is based on the ability of the
antibody to not only bind to an antigen but also bind via its
Fc portion to specialized Fc receptors found on the surface
of a variety of cells such as neutrophils and macrophages.
Antibodies are made by B lymphocytes, which develop
and mature in the bone marrow.

Using immunoelectrophoresis, Grover et al. (1983)
reported having found three types of immunoglobulins
in camels, and their associated subclasses, namely IgG1,
IgG2, IgA and IgM. These antibodies were also reported to
be antigenically distinct from those of other farm animals
and man. Ten years later Azawi et al. (1993) successfully
separated immunoglobulins G and M from camel sera by
ammonium sulfate precipitation, gel filtration and fast
protein liquid chromatography. They did not however find
IgA antibodies. Interestingly, they observed three bands
in gels which reduced disulphide bonds. Two of the bands
corresponded to the typically observed heavy and light
chain antibody molecules but the third was thought to be
unique but was not further investigated.

Hamer-Casterman et al. (1993) reported that in
addition to the heterotetrameric antibodies consisting of
two heavy and two light chains, described in other animal
species, camelids also have a unique sub group of smaller
antibodies. These were likely those observed by Azawi
et al. (1993) and recognized to be unique but were not
further studied. Hamer-Casterman and his group were
able to demonstrate that these antibodies consisted only
of heavy chains. These antibodies are now appropriately
referred to as heavy-chain antibodies (HCAbs). The heavy
chains, found in these antibodies, are also smaller than
those of heavy chains of conventional antibodies. Their
smaller size is attributed to the lack of the CH1 domain
which functions as the anchor for the light chains. Nguyen
et al. (1999) concluded that the loss of the splice consensus
signal was responsible for the removal of the entire CH1
domain in camel IgG2a HCAbs. The variable domain is
therefore joined directly to the hinge region in HCAbs.
HCAbs correspond to the IgG2 and IgG3 fractions. The
IgG2 fraction (92 KDa) binds only to protein A, whereas
the IgG3 (90 KDa) fraction binds to protein A and protein
G. By differential pH elution IgG2 was shown to consist of
two fractions IgG2a and IgG2b (Muyldermans and
Lauwereys, 1999). IgG1 consists of the classical H\textsubscript{L},
antibodies that bind to protein A and G (De Genst et al.,
2006).

HCAbs are reported to vary but can account for upwards
of 75% of their circulating immunoglobulins (Nguyen et
al., 1998). Of particular interest is the possibility that
camels have likely two lineages of B-cells, each secreting
different sets of V(H) gene segments used by four and
two chain immunoglobulins (Ungar-Waron et al., 2003).
Furthermore, sound evidence has been presented that the
variable domains found in camel HCAbs are encoded by
other genes than those of four chain immunoglobulins
(Nguyen et al., 1998) and raises the likelihood that V(H)
genomes of HCAbs do not arise through an ontogenic process
of somatic hypermutation.

Camel HCAbs, unlike the HCAbs derived from
mouse myelomas or those found in human sera have
been shown to be not only functional but also have some
very unique characteristics. Camels for example infected
with Trypanosoma evansi mount an immune response
with a repertoire of antibodies consisting of conventional
antibodies, as well as those made up of HCAbs (Hammers-
Casterman et al., 1993). Camel HCAbs not only react
with a wide range of antigens (Muyldermans and
Lauwereys, 1999) but also interact with certain antigens
differently than conventional antibodies. Camels that were
immunized with bovine red blood cell carbonic anhydrase
and porcine alpha-amylase developed HCAbs that acted
as competitive inhibitors of these enzymes, in contrast to
conventional antibodies that were incapable of binding
to the enzymes’ active sites (Lauwereys et al., 1998).
This ability is ascribed to the difference in the antigen-
binding sites of these different molecules. Whereas
antigen recognition in conventional antibodies results
from the interaction of antigen binding sites contained
within both heavy and light chains, HCAb molecules
have binding sites that are contained within a narrowly
defined region of one molecule, the variable domain of
the heavy chain (Muyldermans and Lauwereys, 1999).
The antigen-binding site of the HCAb is a single variable
domain referred to as the variable domain of the heavy
chain of HCAbs (VHH) (Muyldermans, 2001; DeGenst et
al., 2006; Deschacht et al., 2010). This finding has been
a source of considerable interest as VHHs (15KDa) are
smaller than the antigen-binding sites of conventional
antibodies. Their production is easier than that of normal
mammalian antibodies (Alvarez-Rueda et al., 2007).
They can be cloned as fusion domains to effector proteins
(Muyldermans, 2001) and expressed in bacteria resulting
in quantitative larger amounts. Besides, VHH tend to
retain their ability to bind to the antigens that elicited their
production (Roovers et al., 2007). Of particular interest
is that VHHs are able to recognize small antigenic sites
that conventional antibodies cannot penetrate (De Genst
et al., 2006; Paalinen et al., 2011). Examples of their
potential uses include their development against the
epidermal growth factor receptor (Omidfar, et al., 2007;
Roovers et al. (2007) and nuclear poly (A) binding protein 1 (Verheesen et al., 2006). A recent study by Paalanen et al. (2011) reported on the ability of VHVs to serve as both activators and inhibitors for a protein kinase. This is of particular interest as there are ten isotypes of protein kinase C (PKC). VHVs permit the elucidation of the function of the PKCs and furthermore have potential therapeutic benefits for diseases that result from abnormal PKC signaling such as patients with type II diabetes. It has also been reported by Dumoulin et al. (2003) and Chan et al. (2008) that a camel antibody was able to inhibit the aggregation of lysozyme that is involved in the production of amyloid fibrils, such as those associated with the development of Alzheimer’s and Parkinson’s disease. A very exciting finding has been that VHVs, at least in vitro, were able to inhibit the replication of poliovirus and this raises the hope that they might serve as potential antiviral compounds (Thys et al., 2010). There seems to be an inexhaustible potential for the use of camel VHVs. The DNA for the binding domains can be isolated and they can be generated through bacterial engineering (Arabi et al., 1997) or expressed in mouse cells (Nguyen et al., 2003). Other interesting prospects for these small molecules are their use in immunolabeling of targets such as tumors. They will also be more efficient at penetrating tumors and reaching their specific targets. Also, the single domain antibody might be useful in the development of anti-idiotypic vaccines (Muyldermans and Lauwereys, 1999). There is evidence that these smaller antibodies are less antigenic and thus might not initiate significant immune responses in patients. Herrera et al. (2005) reported that horse and sheep IgGs had more anti-complementary activity than camel IgGs. This is significant as anti-complementary activity is likely a chief mechanism leading to adverse reactions after administrations of heterologous IgG preparations in humans. This would make antisera raised in camels for use against snake or insect venom serum potentially safer than that raised for example in horses (Meddeb-Mouelhi et al., 2003; Herrera et al., 2005; Harrison et al., 2006). Interestingly is also the observation that camel antibodies have been reported to be less antigenic when injected into mice, and unlike horse antibodies, did not cause agglutination of human erythrocytes in vitro (Herrera et al., 2005). Camel VHVs have been reported to sense or induce conformational changes on different isoforms of prostate-specific antigen (PSA) and it was postulated that this might be of potential benefit to study PSA conformational flexibility and to differentiate different stages of prostate cancer (Saerens et al., 2004). The potential use of camelid antibodies is boundless and should help to raise the profiles of camels throughout the world.

The present review highlights some of the most interesting findings pertaining to the camelid immune system and above all is an attempt to prick the curiosity of researchers and spur them on to take note of this once dormant field of immunobiology and gain an appreciation for the immense value of this ancient friend of mankind.

References


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