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

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استعراض لبعض الملاحظات عن معلمات مختارة من نظام المناعة في الإبل

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الخلاصة: بالرغم من وجود الإبل لفترات طويلة مع البشر، إلا أن هناك عدد محدود من الدراسات ذات العلاقة بنظام المناعة في هذا الحيوان. ويعتبر هذا من سوء الحظ إذ أن الإبل قد تطورت عبر التاريخ ليس إلى حيوان قادر فقط على البقاء والعيش تحت ظروف مناخية صعبة للغاية، ولكنه تطور أيضاً إلى حيوان مقاوم بدرجة كبيرة للعديد من الأمراض المعدية. وبالتالي فإنه من المهم محاولة فهم المكونات الفاعلة المختلفة في نظام المناعة في الإبل وذلك لاستخدام أساسياتها كقاعدة ممكنة للتحكم في أنظمة المناعة للحيوانات الأليفة الأخرى حتى يمكن التعامل مع مسببات الأمراض بنفس الكفاءة. تم استعراض الجهود البحثية الحديثة عن أنظمة التكامل والبلعمية بالإضافة إلى أنواع الأجسام المضادة الفريدة التي توجد في فصيلة الجمليات والتي هي مثار اهتمام واسع في الوقت الحالي. كما تم إبراز الدور المهم لوسائل التشخيص والعلاج المحتملة.

كلمات مفتاحية: الإبل ، الجهاز المناعي، المضادات الحيوية ، الخلايا المناعية.

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 into 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.

Introduction

The camel remains to a great extent an enigma shrouded in the mysteries of contemporary biology, despite centuries of man's intensive dependence on and interaction with this species. Of particular interest is the fact that not only can the camel survive under some of the most extreme environmental conditions but it also appears to have evolved into an animal with low susceptibility to a variety of diseases (Fazil and Hoffman, 1981). Undoubtedly, studies endeavoring to elucidate the immunological mechanisms utilized by camels to fight pathogenic agents might lead to important breakthroughs in our understanding of the disease process in general, and perhaps open doors of knowledge that may enhance our ability to stimulate the immune system of man and other animals to function in a similar effective manner.

The immune system is a complex network consisting of many cellular and non-cellular components which interact with each other. For simplicity, it is commonly divided into an innate (non-specific) and a specific immune system. Over the last twenty years a growing body of observations and studies have been undertaken on the camel's immune system and published in a wide spectrum of international journals. The present review is an attempt to consolidate some of the most interesting findings, in the hope that this information might encourage even more scientists to explore and exploit the immunobiology of this unique animal, and equally important, to acknowledge the fine work being undertaken by a handful of researchers in various parts of the world who are helping to bring the study of one of man's oldest companions into vogue.

The Complement System

The complement system is made up of at least twenty different proteins that are present in high concentrations in the blood and tissues. These proteins work together in a cascade fashion

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 parasitemia 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.,* 1977; 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.

Hamers-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_2L_2 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) genes 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 (Hamers-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; Paallanen *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 VHHs 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). VHHs 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 VHHs, 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 VHHs. 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 exciting 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 antiserum 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). Interesting 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 VHHs 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.

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