REVIEW: I –THE ORGANISATION AND FUNCTIONING OF THE IMMUNE SYSTEM: PARTICULAR FEATURES OF THE RABBIT

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ABSTRACT: This article summarises the general organisation of the immune system, and the origins of its cells and their roles. The various aspects of the immune response, innate, specific, humoral and cell mediated, are briefly described. The immune response of the intestinal mucosa is presented with the inductive and effector sites. The regulatory pathways of the immune response and the techniques that can be used to evaluate the immune status of an animal are also presented.

RÉSUMÉ: Revue: I- Organisation et fonctionnement du stème immunitaire: particularités chez le lapin.
Ce texte rappelle l'organisation générale du système immunitaire, l'origine des cellules le composant et leur rôle. Les différents aspects de la réaction immunitaire, naturelle et spécifique, humorale ou à médiation cellulaire, sont abordés. La réponse immune de la muqueuse intestinale, avec présentation des sites inducteurs et effecteurs, est plus particulièrement développée. La régulation de la réponse immunitaire et les techniques utilisables pour évaluer le statut immunitaire d'un animal sont également présentées.

INTRODUCTION

From the embryo stage all animals are subjected to very diverse aggression, particularly those of the multitude of micro-organisms present in the environment. Some micro-organisms are tolerated and others can cause disease if they invade the body or multiply in an uncontrolled way, and an animal has several systems of defence. The first is the non-specific system of defence, i.e. its mode of action is the same whatever the causative agent, and it is involved very quickly after attack. The second is the specific system of defence which is complex and makes it possible to respond in very varied ways according to the causative agent, whether virus, bacteria, fungi, or single or multicellular parasites. These two systems constitute the immune defence system and their synergistic effect generally leads to immunity (protection against a second attack by the same agent). In most cases, the animal's response is effective. However it is possible to act on the immune response, to initiate it or stimulate it (vaccination against some causative agents, fight cancer, immunodepression...) or to attenuate it when it has harmful effects (hypersensitivity, allergies, graft rejection...). We undertook a review of the knowledge concerning the general organisation of the immune system and its functioning, and cite the characteristics the rabbit to provide better understanding of the relationship between nutrition and immunity which will be presented elsewhere in this issue.

GENERAL ORGANISATION OF THE IMMUNE SYSTEM. CHARACTERISTICS IN THE RABBIT

The lymphoid system of the rabbit is organised overall in the same way as that of other mammals. Primary and secondary lymphoid organs can be distinguished. Lymphoid cells circulate between these organs via the blood vessels and lymphatic system.
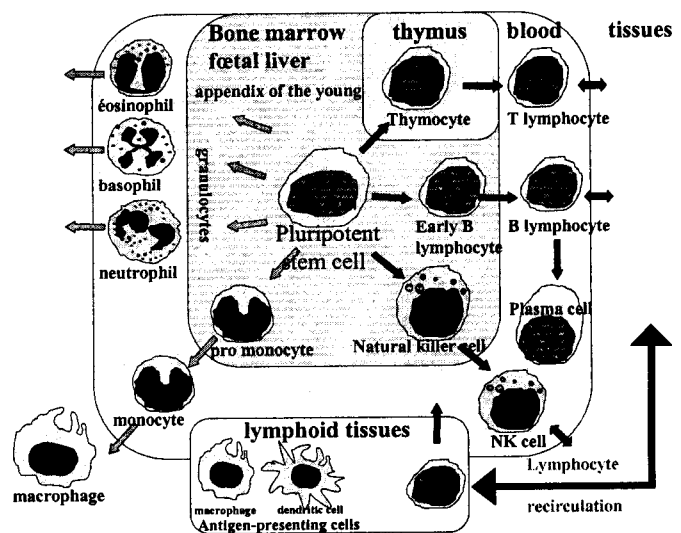

Figure 1: Origin of leukocytes. Differenciation from pluripotent Stem cells.

Origin of the cells of the immune system

All the cells of the immune system are derived from pluripotent bone marrow Stem cells (Fig 1).

These cells are at the origin of three lines. One line ends in the formation of erythrocytes and blood platelets. The cells of the myeloid line differentiate to blood leukocytes, some of which can migrate towards tissues to become mast cells and macrophages ensuring the capture and destruction (phagocytosis) of certain causative agents. B and T lymphocytes and Natural Killer cells (NK)/Large Granular Lymphocytes are from the lymphoid line. B and T cells are characterised by the way in which they ensure protection of the body against attackers. B cells produce antibodies or immunoglobulins and T cells act by destroying infected cells or by cooperating with the other cells of the immune system.

Finally leukocytes present exclusively in the lymphoid organs specialise in collecting antigens and in their presentation to T cells: these cells called dendritic cells (Langerhans cells in the skin...) are derived from unidentified stem cells.

Primary and Secondary lymphoid organs (Fig 2)

- Lymphoid line cells differentiate and become mature in the primary lymphoid organs, i.e. bone marrow, thymus and foetal liver. Lymphocytes acquire the capacity to synthesise molecules during development which make it possible to identify subsets (e.g. surface cell markers and cell receptors named CD4, CD8...). The B cells differentiate completely in the bone marrow whereas the T cells finish their maturation in the thymus. A characteristic of the rabbit is that the vermiform appendix, a lymphoid organ belonging to the intestine and located
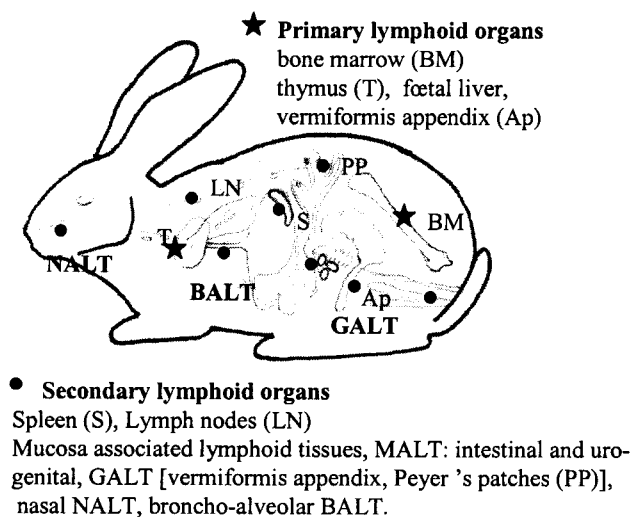
at the end of the caecum, has the role of a prima lymphoid organ in the production and maturation of cells during first weeks of life (REYNAUD et WEII 1996 ; POSPISIL et MAGE, 1998).

- After differentiation, lymphocytes migra from the primary lymphoid organs towards secondary lymphoid organs, i.e. the spleen, lym nodes and lymphatic tissue developed within t mucous membranes (Mucosa Associated Lympho Tissue or MALT), in which the lymphoid cells a stimulated and proliferate.

The spleen is involved in the destruction senescent erythrocytes and platelets and the white pu which constitutes the lymphoid part of the organ ha: role in the immune response. The lymph nodes ensu filtration of interstitial body fluids and of lym during its passage from organs towards the thorac duct. All the organised lymphoid organs have similar, heterogeneous and compartmentalis structure: zones of primary follicles rich in B ce surrounded by areas rich in T cells, zones of seconda follicles rich in B cells at various stages of maturati (from the lymphoblast to the plasmocyte) and reticul tissue containing phagocytes and antigen-presenti cells.

All the mucous membranes of the body ha associated lymphatic tissue (MALT) whose first rc is to ensure defence of the host against pathogens ai to ensure protection of the mucous membranes l controlling the inflammatory response. Nas Associated Lymphoid Tissue is associated with tl upper respiratory tract, Bronchoalveolar Associate Lymphoid Tissue with the lower respiratory tract ai Gut Associated Lymphoid Tissue with the gastr intestinal and urogenital tracts. The rabbit lympho system associated with the intestinal mucosa has oi main characteristic, the considerable development the vermiform appendix.

This organ, whose role is that of a primai lymphoid organ in the young rabbit, functions as secondary lymphoid organ in the growing animal ar thus intervenes in induction of the specific immur response. B cells, which differentiate in the append: in contact with the intestinal flora (FUSCHIOTTI *et a* 1997 ; LANNING *et al.*, 2000), then migrate to oth parts of the intestine, in particular to Peyer's patche The appendix is essential for diversification of tl antibody repertoire and for development of tl mucosal immune system (DASSO and HOWELL, 1997 VAJDY *et al.*, 1998).
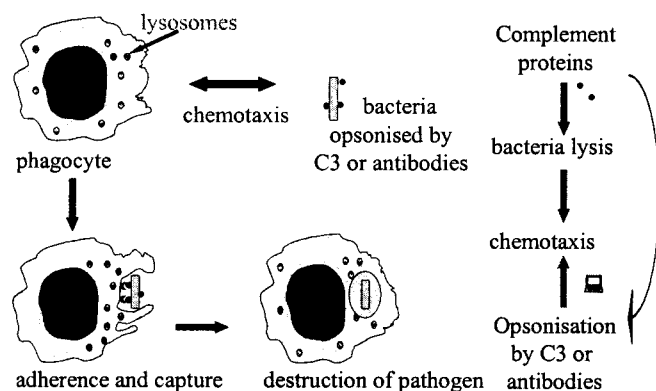

★ **Primary lymphoid organs**
bone marrow (BM)
thymus (T), fœtal liver,
vermiformis appendix (Ap)

• **Secondary lymphoid organs**
Spleen (S), Lymph nodes (LN)
Mucosa associated lymphoid tissues, MALT: intestinal and uro-
genital, GALT [vermiformis appendix, Peyer 's patches (PP)],
nasal NALT, broncho-alveolar BALT.

Figure 2: Distribution of primary and secondary lymphoid organs in rabbit.

Figure 3: Triggering of the immune response by phagocytosis.

GENERAL INFORMATION ON THE IMMUNE RESPONSE

It is necessary to distinguish the "innate" or "natural" primary immune response which is non-specific, representing the first line of defence against an attacker, from the adaptive or acquired immune response which is directed against a specific foreign element in the body. The immune system has a whole range of mechanisms to destroy pathogenic micro-organisms, each one being adapted to a particular type of micro-organism. The duality of these two systems of defence is only apparent because they often act as synergistic systems.

The innate immune response generally begins with an acute general inflammatory reaction; the cells of the immune system are distributed throughout the body and in the event of aggression, effector cells of the immune system must be able to accumulate within the site of attack. Effector cells are a group of leukocytes formed by phagocytes, i.e. blood monocytes and polynuclear neutrophils. Several proteins present in the serum, i.e. acute phase proteins, C reactive protein (CRP) and proteins of the Complement system (about twenty proteins acting as a cascade) are set in action. These molecules interact between themselves and with other elements of the immune system (Fig 3). For example, many micro-organisms spontaneously activate the Complement system by the "Alternative pathway"; this reaction is nonspecific because it does not require the formation of immune complexes. At the end of the reaction, the micro-organism covered with proteins of the Complement will be captured by the phagocytes in a sequential process. The micro-organisms or particles become attached to the phagocyte surface, the surface invaginates and the cell membrane completely surrounds the particle or pseudopodes move outward and surround the particle, forming a phagosome

(phagocytic vesicle). Polynuclear neutrophils die after having phagocyted the particles while the mononuclear phagocytes (monocytes in the blood) migrate towards tissues and become tissue macrophages. These cells very effectively present the processed products of the micro-organisms (antigens) to T cells and thus take part in the specific reaction; in response, T cells secrete soluble factors (cytokines) which stimulate the phagocytes and allow them to destroy the phagocyted micro-organisms.

The specific reaction (Fig 4) begins with preliminary recognition of the foreign element, whether it is present in the host cells (virus, bacteria or intracellular parasites) or in the extracellular fluids; it is a complex whole of interactions, bringing into play the membrane receptors of the cells and in particular the molecules of the Major Histocompatibility Complex (MHC), molecules characteristic of the individual, and involved in particular in the rejection of organ transplants... Complement proteins can also be activated by the classical pathway after adhesion of antibodies to the micro-organism; in contrast to the alternative pathway, the classical pathway is a specific reaction brought into play after formation of an immune complex. The specific immune response is thus two-fold.

- Humoral immune response: stimulated B cells multiply, some transform into "memory cells" able to multiply at the time of subsequent contact, and the majority of stimulated B cells are transformed into plasmocytes secreting various types of antibody (each cell secretes only one type of antibody, IgM, IgG, IgA, IgE in case of allergy, and more rarely IgD).

The first immunoglobulins secreted are IgM, and then the levels of IgG and IgA increase. When a second contact with the infectious agent occurs, IgG secretion is much faster and higher. The immunoglobulins initially play the role of specific antigen receptors (recognition and binding). After binding to the antigen, the immunoglobulins can activate the enzymatic cascade of the classical complement pathway or a complex set of interactions with various cell types. These two methods lead to the destruction of the antigen.

During gestation, immunoglobulins can be transmitted to the foetus via the placenta by the amniotic fluid; for the doe rabbit this occurs at the end of gestation, when the placenta becomes haemochorial. Immunoglobulins are also transmitted to the young by the colostrum then to a lesser extent
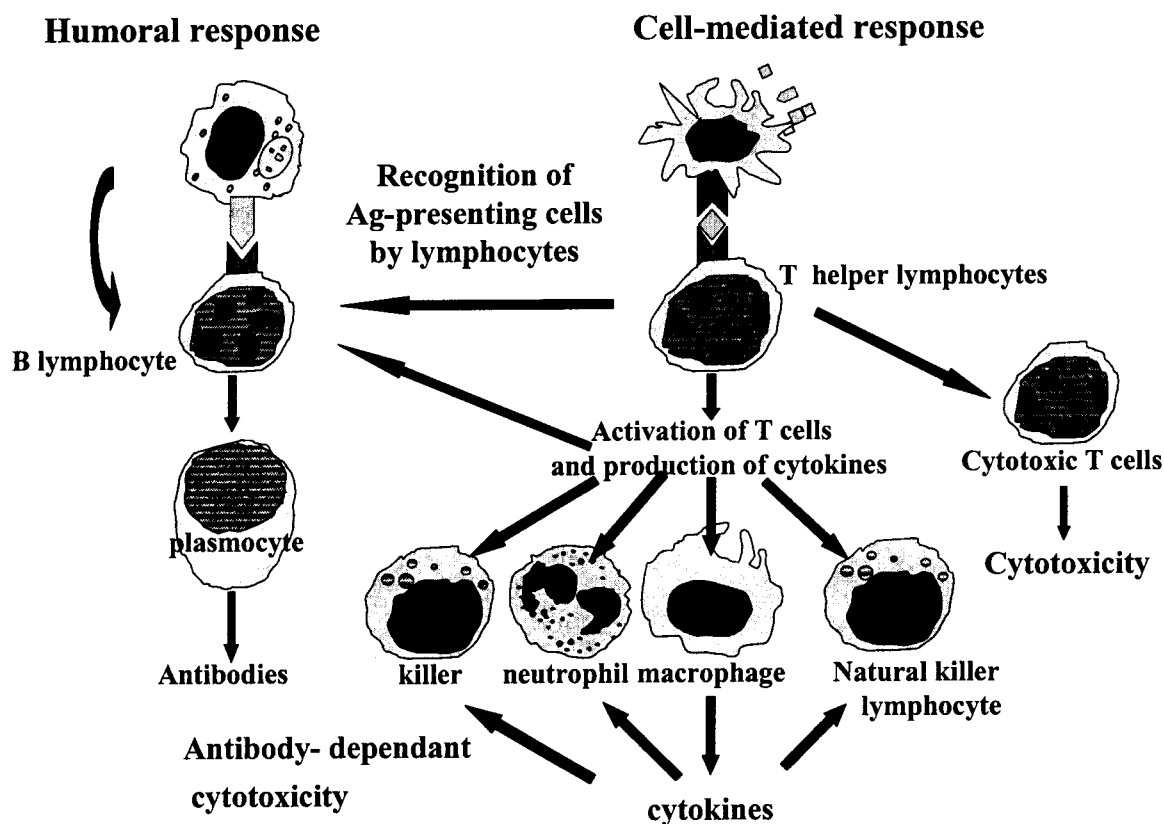
Humoral response

Cell-mediated response

Figure 4: The specific immune response: main pathways and cell interactions.

by milk: the value of IgG is directly related to absorption through the intestine but IgA protect the mucosa against aggressor agents present in the intestinal lumen. The protection thus conferred is known as "passive immunity". This passive immunity has the advantage of protecting the young animal from attack by causative agents in the mother' s environment and therefore its own, but has the disadvantage of delaying the setting up of an active response, whether spontaneous or caused by vaccination.

- Cell-mediated immune response: this response is complex and brings into play processes of recognition between cells (via surface receptors) and of interaction between cells by the production of substances called cytokines. There are four families of cytokines : interleukins (ILs), tumour necrosis factors (TNFs), interferons (IFNs) and colony-stimulating factors (CSFs). The principal cells involved in cell-mediated immune response are T cells. They act either by causing the destruction of certain cells, as is the case with T-cytotoxic lymphocytes (most often CD8+), or through co-operation with other T cells, B cells or phagocytes, as is the case with T helper cells (Th), generally CD4+.

B and T cells interact with the phagocytes. The majority of the reactions against causative agents

therefore bring into play elements of natural immunity and specific immunity at the same time. At the initial contact, natural immunity prevails but the specific response is started simultaneously. Thus, at the time of the second contact with the same micro-organism, cells known as "memory cells" are able to respond in a specific way more quickly and more effectively. Lymphocytes circulate permanently in the body *via* the lymph and blood. This circulation increases the probability of the primed lymphocyte meeting its specific antigen and allows the activated lymphocyte (effector cell or cell memory) to migrate from the site of induction of the immune response (often a site of penetration of pathogens into the body) towards the corresponding effector sites. The capacity of the lymphocytes to return to tissues involved in the presentation of the antigen and their activation has been known for a long time; this phenomenon is known as homing. Thus, intraepithelial lymphocytes (IEL) isolated from the intestine and injected intravenously have the capacity to return preferentially to the intestine and GALT (SYDORA *et al.*, 1993; BUZONI-GATEL *et al.*, 1999). Non-activated and activated lymphocytes express different recognition molecules and, whereas the non-activated lymphocytes can migrate towards many lymphoid organs (O'NEILL *et al.*, 1992), activated lymphocytes migrate preferentially towards their site of activation (POTSCH *et al.*, 1999).

Lastly, the immune response can sometimes present harmful characteristics for the animal. The body may not recognise as foreign certain elements not forming part of itself; this is known as the tolerance phenomenon. For an individual in good health, the tolerance phenomenon is the rule for nutrients and saprophyte flora. In contrast, rejection reactions may occur which can evolve to allergic type reactions. Moreover sometimes the body recognises elements of itself as foreign and in this case auto-immune disease can develop.

INSTALLATION OF THE SPECIFIC IMMUNE RESPONSE. EXAMPLE OF AN INTESTINAL INFECTION

Elements foreign to the body (apart from causative agents transmitted by sting or bite) penetrate either through the skin or the mucous membranes. As we have seen, the majority of cells involved in natural immunity also play a role in the adaptive immunity. We will take the example of the immune response against a causative agent developing in the intestine.

The intestinal mucosa is a significant exchange surface with the external environment, through which nutrients are absorbed. It also constitutes a physiological and immunological barrier against a great number of micro-organisms and foreign substances. The regulation of the immune response in the intestinal mucosa is particularly complex. On the one hand, the variety of antigens in the intestine is enormous and tolerance mechanisms must be induced with respect to the majority of antigens. In addition, it is necessary to set up non-specific and adaptive immune mechanisms during invasion by a micro-organism. Under normal physiological conditions, a homeostatic balance is maintained and the inflammatory processes are controlled.

Several non-immunological factors make it possible to ensure the defence of the mucous membranes: the resident digestive saprophyte flora and secretions create an unfavourable environment for the growth of causative agents. Intestinal peristalsis, the mucus and the glycocalyx make it possible to reduce the interactions between causative agents and epithelial cells. Some substances (lactoferrin, lactoperoxidase and lysozyme) can have an inhibiting effect on the development of causative agents. Lastly, the gap junctions, which ensure cohesion between epithelial cells, prevent the intercellular passage of causative agents.

When these mechanisms do not allow elimination of the causative agent, immune defence

mechanisms can be set up. GALT alone contains more immune system cells than the remainder of the body. It can morphologically and functionally be divided into 2 parts: the inductive sites of the immune response (places of recognition and / or capture of attacking agents and of activation of cells starting the reaction against antigens) are Peyer's patches plus, in the rabbit, the vermiform appendix and the *sacculus rotundus* which constitute the organised lymphatic tissue; the effector sites of the immune response (places where the cascade of interactions between cells ends in the neutralisation or destruction of the attacking agent) are the diffuse lymphatic tissue of the *lamina propria* (tissue located under the epithelium) and lymphocytes of the intestinal epithelium.

Inductive sites of the immune response of the intestinal mucosa

The lymphatic tissue of Peyer's patches, the vermiform appendix and the *sacculus rotundus* consist of lymphoid follicles, surmounted by a dome and specialised epithelium (Follicle-Associated Epithelium, FAE), separated by interfollicular zones. The lymphoid follicles comprise a germinative centre and a corona which contain primarily B cells producing IgM, and also macrophages and CD4-Tcells (ERMAK *et al.*, 1994).

The interfollicular zones are rich in CD4 and CD8 T cells and macrophages. It is at this level that blood lymphocytes penetrate the mucosa.

The Follicle-Associated Epithelium is a very specialised epithelium. It differs from the intestinal epithelium by the absence of mucus cells, the absence of secretion of the dimeric IgA receptor (PAPPO et OWEN, 1988) and of alkaline phosphatase activity (OWEN et BHALLA, 1983) by its capacity to bind lectins (NEUTRA *et al.*, 1987) and by the presence of large numbers of particular epithelial cells, M cells, which in the rabbit account for 50% of the cells of the epithelium (PAPPO, 1989).

M cells are characterised by the presence of atrophied and irregular microvilli and by a sparse glycocalyx and mucus layer facilitating access to their apical membrane by luminal antigens (OWEN and JONES, 1974). Their basolateral membrane forms a pocket in which lymphocytes and macrophages are embedded. M cells collect a wide variety of antigens and micro-organisms and make them available to these lymphoid cells.

The activated T cells present in the FAE have a particular phenotype (ERMAK *et al.* 1990, 1994, 1995). They are believed to be dendritic cells (WEINSTEIN

and ANDERSON, 1998).

Capture of the antigens by macrophages or dendritic cells of the domes and follicles allows activation of lymphocytes and induction of the specific immune response.

Effector sites of the mucosal immune response

Apart from the organised lymphatic tissues, most lymphoid cells are distributed in the *lamina propria* and the epithelium of the intestine. The effector immune response can thus develop throughout the intestinal tract.

The epithelium is composed mainly of four cell types derived from pluripotent cells in the crypts (zones of cell proliferation at the base of villi) and which take part in the restoration and the maintenance of its integrity (GEBBERS and LAISSUE, 1989). Of these, Paneth cells, localised in the crypts, secrete granules rich in lysozyme with a strong bacteriolytic activity and antibiotic peptides such as the cryptidine; mucus cells contribute to the formation of a protective coating on the surface of enterocytes.

Enterocytes (90% of the cells of the intestinal epithelium) are absorbing cells but are also involved in the immunising mechanisms of protection. These cells ensure the transfer of IgA synthesised by plasmocytes of the *lamina propria* until the intestinal lumen. IgA, which are antibodies prevalent on the surface of mucous membranes, prevent the attachment of micro-organisms and luminal antigens to the epithelium (GEBBERS and LAISSUE, 1989). Enterocytes also express MHC molecules allowing a specific response, and are able to produce some cytokines.

According to GUY-GRAND and VASSALLI (1993) 90% intraepithelial lymphocytes (IEL) localised between the enterocytes are T-CD8 type. The presence of intracytoplasmic granulations rich in perforin, granzymes and Fas-Ligand is evidence of cytotoxic activity of. the IELs (GUY-GRAND et al., 1996).

Lastly, the underlying part of intestinal mucosa is made up of a layer of connective tissue forming the chorion or *lamina propria* containing very numerous lymphoid cells (about 50%). The T cells present at this site primarily have the function of helper cells (AKBAR et al., 1988 ; TROUT et LILLEHOJ, 1996).

Triggering the intestinal immune response

The first barrier met by foreign agents entering the body via an intestinal route is the epithelium. The enterocytes are the first cells involved and would trigger the immune response. The macrophages, dendritic cells and NK cells also play a significant role.

The principal inductive sites of the immune response in the rabbit are Peyer's patches and the vermiform appendix. The natural type of immunity in these organs is type Th2 (production of IL-4, IL-5 and IL-10) and Th3 (production of TGF-b) and leads to a cytokine pattern in favour of the production of IgA and the stimulation of cells inducing oral tolerance of the antigens. At the time of an attack, homeostasis is broken; the imbalance which is created involves the switching of the immune response towards a Th1-type pattern (production of IFN- g and IL-2) and the production of inflammatory cytokines.

The non-specific immune mechanisms lead to the activation of lymphocytes and will thus start the process of specific immunity.

- The humoral response induced by a primary infection is characterised by the production of specific type IgM, IgA and IgG antibodies in the serum. The appearance of IgM and IgA precedes that of IgG but does not persist. IgG are detected later and production is maximum two to three weeks after infection. After a second infection, only the production of IgG increases again and more quickly. However, the antibodies are not always protective, as is the case in coccidiosis (DROUET-VIARD et al., 1996).

- The cellular immune response involves a synergistic action of the two subsets of T-CD4+ and T-CD8+ lymphocytes. CD4+ lymphocytes recognise the antigen associated with MHC class II molecules whereas CD8+ lymphocytes recognise the antigen associated with MHC class I molecules. According to the types of cytokines which they produce during an infection, CD4+ lymphocytes or T helper (Th) will direct the response towards:

i) either humoral mediated immunity (production of cytokines of the Th2 type : IL-4, IL-5 and IL-10) and activation of B cells.

ii) or cell-mediated immunity (production of cytokines of the Th1 type: IFN- g and IL-2) and activation of the cytotoxic functions of macrophages and CD8+ lymphocytes. In the majority of parasitic infections the response is of Th1 type, and in particular production of IFN- g plays a fundamental role in protection. The role played by cell-mediated immunity in the acquisition of resistance to infections by *Eimeria* (parasitic protozoan causing coccidiosis) has been

fully studied in the mouse and chicken and recently in the rabbit (RENAUX et al. 2001). To roughly summarise, in the event of Eimeria infection, the CD4+ lymphocyte subset is more often involved in resistance to the first infection and the CD8+ lymphocyte subset is more involved in resistance to reinfection.

REGULATION OF THE IMMUNE RESPONSE

The early synthesis of inflammatory cytokines and IFN- g by specific or non-specific mechanisms is an essential element in protection against infections. However, the inflammatory response, which involves harmful processes for the causative agent, can also become harmful for the host if they are not controlled (LIESENFELD et al., 1996). Various regulatory mechanisms of the immune response are involved at systemic and local levels to bring the response back to a balanced state.

The antigens themselves, the amounts, administration route and the nature of the cells which present them to the T cells have a significant role in their tolerance or rejection. Immunoglobulins can exert a negative feedback effect on their own production. Lymphocytes also exert a regulatory effect: according to the type of cell stimulated, either the cell-mediated response or the production of antibody will be favoured. Moreover individual factors, neuroendocrine factors (stress) and genetics, in particular the genes belonging to the MHC, participate in regulation.

The enterocytes responsible for the release of the immune response in the intestinal mucosa are also involved in the regulatory pathway of the inflammatory processes. In effect, their capacity for presentation of the antigen seems limited (BARRETT et al., 1993) They seem to be able to activate a subset of CD8+ T-suppressor lymphocytes involved in the regulation of the immune response (CAMPBELL et al., 1999). Epithelial cells can also inhibit the proliferation of IELs and the production of cytokines by these cells (YAMAMOTO et al., 1998). IELs are also involved in the regulation of the inflammatory response (KASPER et BUZONI-GATEL, 2001) as these activated lymphocytes are able to produce great quantities of TGF- b and to reverse the processes of hyperinflammation. IL-10 is a significant cytokine in the maintenance of homeostasis during the response to an oral infection (GAZZINELLI et al., 1996). The type of cell producing this cytokine in the intestine has not yet been determined. Systemic mechanisms of regulation of the immune response are installed subsequently. Thus, the nitrated derivatives (NO),

inflammatory cytokines and IFN- g are able to induce a negative feedback effect on the proliferation of splenic lymphocytes (CANDOLFI et al., 1994) and on macrophage activity (CHANNON and KASPER, 1996).

ASSESSMENT OF THE IMMUNE STATUS OF AN ANIMAL

Given the complexity of the organisation of the immune system of vertebrates, the number of cell subsets involved and numbers of intermediate or effector molecules solicited during the immune response, no single measurement can characterise the immune status of a population overall or of an individual or predict its resistance to an infection. However, various tests make it possible to evaluate the humoral immune and/or cellular, systemic and/or local (mucosal for example) response of an individual (or groups of individuals which are genetically very homogeneous) against a given causative agent. These tests are described in detail in books on basic immunology (ROITT et al. , 1997; JANEWAY and TRAVERS, 1997; etc.).

The first examination to be carried out is the enumeration of blood leukocytes and evaluation of the proportion of each category of cells (blood formulae).

Evaluation of humoral immunity

The humoral immune response is usually measured by assay of antibodies in the serum. Their isotype is generally determined by immunoprecipitation. When the antigen is known, the immunoglobulins produced are assayed with immunoenzymatic techniques (ELISA): the antigen-antibody complexes formed are then bound to an enzyme-conjugated antibody causing a coloured reaction whose intensity is proportional to the quantity of antibody that is bound.

The number of B cells producing antibodies can also be determined by the method of cell lysis plaques in presence of sheep red blood cells and of Complement.

Characterisation of cell-mediated immunity

In vivo cutaneous tests of delayed hyper-sensitivity constitute the best method of evaluation of cell-mediated immunity in human medicine. Antigens such as the tuberculin are injected intradermally and the local reaction is read 48 to 72 hours later. A positive test means that cell-mediated immunity is normal. The other tests intended to evaluate T cell-mediated immunity are complex; in effect, responses

of T cells require an interaction between two cells, a target cell expressing the specific antigen in the form of a peptide-MHC complex and an effector T cell displaying the receptors which recognise the cell surface antigen of the target cell. Three tests can be performed to assess this type of immunity:

i) Enumeration of the various T–cell subsets. To perform these tests, relatively purified suspensions of leukocytes must be prepared. It is possible to measure the relative proportion of each type of lymphocyte and to check if there are any deficits or excesses. This measurement is performed after labelling the various types of lymphocyte with specific antibodies (recognition of their cell-surface antigens) including antibody coupled with molecules emitting fluorescence. The flow-cytometer makes it possible to count the fluorescent cells compared to the cells that are not. In experiments it is possible thus to follow the fluctuations of each cell subset in the blood and various organs (spleen, lymph nodes, mucous membranes...).

ii) In vitro exploration of the functional capacities of the T cells, i.e. their capacity to multiply in the presence of a specified antigen or of various molecules called mitogens starting the cellular division, and the function of cytotoxicity against infected or tumoral cells (this last technique being performed only with cells of histocompatible animals).

iii) Assay of the cytokines produced by activated T cells, IL-2 and IFN γ. Many molecules playing a key role in the communication, proliferation and differentiation of the cells of the immune system are produced during the immune response. The production of these cytokines can be quantified by ELISA test (for extracellular cytokines) or by flow cytometry (for intracellular cytokines); for this purpose the cytokines must be purified and their corresponding antibodies produced. These tools currently do not exist for the rabbit. Determination by RT-PCR or northern-blotting of the level of mRNA coding for the cytokines is a method which can be used to appreciate the degree of stimulation of the cells required to produce a cytokine; this indirect technique is very laborious and for the moment cannot be used as a diagnostic tool.

Other tests

Functional tests can also be performed on phagocytes . Their phagocytic activity (analysed by the incubation of cells in the presence of adequate biological material), their chemokinetic activity (morphological modifications which precede movement, and cell migration towards chemotactic attractant molecules in a Boyden's chamber), their lysosomal activity and the production of cytokines (assay of IL-1, IL-12...) can be tested.

It can be useful to measure the activity of Complement proteins by ELISA tests to assess inflammatory type responses.

Within the framework of studies on the specific mechanisms of local reaction to a causative agent, the distribution of lymphocyte subsets can be visualised *in situ* after labelling of the various categories of cells directly on histological sections (RENAUX *et al.,* 2001). These methods are commonly used but at present exclusively in research.

CONCLUSION

The two types of immune response are interdependent and complementary. Many tests are available to evaluate the immune status of an individual; each test has significance in a given context of aggression. For example in the case of rabbit coccidiosis, it is not very valuable to evaluate the humoral response since cell-mediated response plays a dominating role in the resistance of animals to re-infection. On the other hand, in the case of a bacterial or viral infection, the humoral response should be studied. In experimentation, given the variability of individual responses, and in spite of the laboriousness of the implementation of certain tests, even on homogeneous batches of animals (age, weight, treatments etc...) it is necessary to carry out the tests on a minimum of 10 animals.

Some antibodies specific for several rabbit lymphocyte subsets have been obtained (KNIGHT and LANNING, 1998). However, the current difficulty for immunological research on rabbits lies in the still limited number (in the trade) of monoclonal antibodies recognising the various lymphocyte subsets, and in the absence of specific reagents to measure the production of the different cytokines. These tools must be developed to perform researches in immunopathology of the rabbit more effectively and to for optimal study of effects of certain nutrients on the immune status of the rabbit.

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