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RECENT ADVANCES IN THE IMMUNOLOGY OF PARASITIC INFECTIONS

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INTRODUCTION

Some 30 years ago the first practical commercial vaccine against a parasitic infection was developed. It consisted of irradiated infective larvae of *Dictyocaulus viviparus*, the bovine lungworm, which prior to the development of the vaccine had been responsible for severe morbidity and mortality in calves (Jarrett et al, 1958). That vaccine is still being marketed successfully and several million animals have now been protected by it. There have been very few animals showing side effects to the vaccine. At the time of its development, hopes were high for a similar approach to several other species of parasites responsible for major disease in man and animals. These included diseases such as malaria, trypanosomiasis and schistosomiasis of man, gastro-intestinal parasitisms of domestic animals, fascioliasis and larval cestodiasis of domestic animals. However, in no case was an effective practical vaccine developed. A variety of reasons were responsible for this failure: immune unresponsiveness of young animals, immunosuppression, lack of sufficient immunizing stages or in the majority of cases a lack of knowledge of the important immunising antigens.

However, with the advent of molecular biological techniques, the outlook for practical field vaccines for parasitic infections has changed dramatically and now there are a number of parasitic infections where vaccines constitute realistic approaches to control and are in the final stages of development.

There is an increasing need to develop vaccines as an alternative to chemotherapy. The occurrence of parasitic resistance to anti-parasitic compounds is becoming more common and in some helminth infections of ruminants resistance to the regularly used anthelmintics is serious enough to compromise livestock production. A further point regarding chemotherapy is the increasing consumer opposition to the use of continuous medication for food producing animals and vaccination is a logical answer to such criticism.

Whereas the development of a field vaccine is the practical outcome of immunological studies, such information can also be applied to the diagnosis of parasitic infections, ursurping the classical approaches to diagnosis such as faecal egg counts or the demonstration of parasites in blood, in other body fluids or tissues.

VACCINE DEVELOPMENT

Several parasite vaccines are now under development. Earlier attempts to produce antiparasite vaccines have been frustrated either by the inability to identify the important antigens or the limited availability of antigens responsible for resistance. Organisms were not readily cultivated *in vitro* and in some cases the source of such material (e.g man) made vaccine exploitation impossible. Gene cloning techniques now offer a unique opportunity to

overcome these problems. Specific antibody probes are being developed to identify protective antigens and these in turn are used to identify protective epitopes in microoganisms containing recombinant DNA molecules coding for protective antigens.

Examples of such vaccines are:

Tick vaccines:

After repeared infection with ticks animals develop immunologically mediated resistance to futher infection (Trager, 1939). This often is not complete and is stimulated principally by salivary gland antigens of which a 20 KDa protein, in the case of *Amblyomma americanum,* was recognised as a major immunising component. However,an alternative approach is to immunise with tick gut antigens (Willadsen and Kemp, 1988), these representing concealed antigens, not normally "seen" by the host immune system during normal infection. Antibodies raised by such concealed antigens damage the gut cells during tick feeding to such an extent that host blood appears in the tick haemolymph giving the tick a distinctive red colour. The immunological mechanisms is antibody mediated and is complement independent. As little as seven nanograms of tick gut protein have been shown to be sufficient to immunise an animal.

A recent exciting development is the cloning of membrane-associated midgut derived protein in from *Boophilus microplus* that is immunoprotective in the bovine (Opebeeck *et al,* 1988). This has provided the basis for a genetically engineered vaccine which is now under development in Australia.

It should be noted that since concealed antigens are used for this approach it is unlikely that immune evasion mechanisms associated with salivary gland antigens (Stewart, 1983) will be invoked, nevertheless field challenge will fail to boost the immune response to concealed antigens and regular immunising doses will be required . This indicates that expression vectors, such as the vaccinia virus, may be required to maintain an adequate level of immunity.

Vaccination against Blowflies

Blowfly strike, caused by *Lucilla curprina* might be thought to be an unusual candidate of vaccination studies. Strike or cutaneous myiasis is resposible for some $150m loss annually in Australia alone and control while achievable with insecticidal drugs and sprays suffers from the development of insecticidal resistance by blowflies. Early studies demonstrated that immunisation with homogenates of maggots inhibited subsequent larval growth and excretory-secretory preparations from larvae were shown to induce significant immunity. However, a major development followed the use of gut antigens of *L. curprina* larvae in much the same way as with the tick *Boophilus* (Sandeman, 1990).

Vaccination against Cysticercosis

Several workers have demonstrated that strong immunity can be induced in animals serving as intermediate hosts for important cestodes. The economically important *Taenia saginata* of man causing bovine cysticercosis is amenable to vaccinataion and preparations of tapeworm oncosphere will immunise cattle against infection and also result in passive protection of calves born to vaccinated mothers (LIoyd & Soulsby, 1976). Because of the difficulty of obtaining human derived tapeworm material attention was directed to heterologous sources of immunogens and while some useful protection was achieved with cross reacting cestodes, none was so effective as the homologous parasite (Lloyd, 1979).

Work with the rodent species *Taenia taeniaeformis* showed that excellent immunity could be induced with oncospheral material, that protective antibody was IgA in nature where maternal immunity was concerned but was IgG based in the adult animal (Lloyd and Soulsby, 1978).

Such initial studies paved the way for work with *Taenia ovis*, an economically important parasite of sheep in Australia and New Zealand. Antigens in the range of 47-52KD from *T. ovis* eggs were shown to be protective in sheep. Hatched and activated onocospheres of *T. ovis* were used as a source of mRNA for the construction of a cDNA library. This was constructed in lambdagtll and two fusion proteins β-gal-45S and β-gal-45W purified from selected clones were tested in vaccination trials. While sheep immunised with the β-gal fusion proteins produced antibodies which reacted with oncosphere antigens of 47-52KD, no protective immunity was induced.

A different expression system was more successful and using a plasmid vector which expresses antigens as fusion proteins with the enzyme glutathione S-transferase of *Schistosoma japonicum* (pSj 10ΔBam 7 stop 7, a precursor of the pGEX-1 vector) the 45W and 45S cDNA were cloned into this vector ant the fusion proteins GST-45W and GST-45S proved very efficient in inducing protective immunity in sheep when injected with saponin adjuvants (Johnson et al, 1989).

Why a β-gal fusion failed to immunise while a GST fusion did is unclear.

These studies have led to the field trial of a commercial vaccine and provide the basis for similar vaccines for bovine and swine cysticercosis and also hydatid infection in man and animals.

Vaccination against Tick Borne Protozoa

A vaccine against *Babesia* consisting of blood containing an attenuated strain of *Babesia* has been available for some time (de Vos *et al*, 1987). This induces a mild infection resulting in immunity to more severe field challenges. The disadvantages of the vaccine are that as a living agent it has a limited shelf-life and on occasions may induce more severe responses in vaccinated animals. Subunit *Babesia* vaccines are being pursued for *B. bovis*, *B. bigemina* and *B. divergens* and a 42 KD immunodominant protein appears to be particularly important source of immunogen as well as for a diagnostic reagent (Goff *et al*, 1988).

Several sporozoite antigens of *Theleria parva* and *Theileria annulata* are candidate molecules for immunisation. A 67 KD component has been identified as important in protection and has been expressed in *E. coli* as a C-terminal fusion protein with a *Schistosoma japonicum* antigen Sj26 using the pGEX expression vector system (Smith & Johnson, 1988). With the *Theileria spp.* the role of T cells is particularly important and in the case of the 67KD antigen, helper T-cell epitopes should be present (Good *et al*, 1987). An approach using live recombinant vectors may be important with organisms such as *Theileria* and delivery systems such attenuated strains of *Salmonella typhimurium* or vaccina virus are under investigation (Smith *et al*, 1984).

IMMUNOLOGICAL MECHANISMS OF PARASITES EXPLUSION

Despite some 50 years research with a range of parasitic helminths the mechanisms by which parasitic helminths are expelled from the host are largely unknown. Expulsion from the alimentary tract is of particular concern in the case of gastro-intestinal nematodes of domestic ruminants.

In general, immunologically mediated expulsion of parasites is specific in nature, and burdens of different species of nematodes for example can exist in an animal immune to one of the species. However, probably due to non-specific factors induced by immunological reactions there may be apparent cross reactions resulting in expulsion of a different species. Thus acute expulsion of *Haemonchus contortus* (self cure) from the abomasum can result in a concomittant loss of *Trichostrongylus spp.* in the small intestine and other abomasal worms,

but immune explusion of intestinal worms did not result in loss of the abomasal species (Stewart, 1953, 1955).

Mast cells:

Mast cell hyperplasia is a feature of many nematode infections (Rothwell, 1989) and is accompanied by increased vasoactive amine levels at the site of infection (Miller, 1971). Thus high levels of histamine and 5-HT occur in parasitised mucosae but reach a peak after worm expulsion in some cases. Hence the true role of mediators in expulsion has yet to be fully clarified.

The interrelationship between mast cells and lgE, a common immunogloblin in helminth infections, which by cross-linkage of mast cell membrane bound lgE by specific antigen, results in mediator release, is accepted as an important component of rejection (Ogilvie, 1970). However, other cells may release amines (e.g. basophils, platelets and enterochromanffin cells) and these may also be armed by lgE and secrete vasoactive amine when challenged by specific antigen. Mast cell deficient animals vary in their capability to expel various parasites but on the whole expulsion is delayed in such animals (Uber et al, 1980).

It has not been possible to demonstrate any direct effect of vasoactive amines on nematodes *in vitro* and the still likely explanation of worm loss is the indirect effect of amines on the microenvironment of the parasite, which in addition is heavily infiltrated with various cell types including mast cells, eosinophils and goblet cells.

Eosinophils:

These are well known cells in parasitised tissues. A variety of functions have been ascribed to them including their attraction to amines released by mast cells by eosinophil chemoactive factor of anaphylaxis (ECF-A) with subsequent down-regulation of vasoactive amines produced by mast cells. The frequency of eosinophils in tissues in association with dead or dying parasites has suggested that eosinosphils may be responsible for the death of the organism (Mackenzie, 1980). Indeed, it is possible to demonstrate eosinophil mediated destruction of some parasite developmental stages through the action of major basic protein (MBP) and eosinophil cationic protein (ECP) (McLaren *et al*, 1977). However, direct contact between eosinophils and intestinal lumen dwelling nematodes is not a feature of immune expulsion.

Goblet cells:

Goblet cell hyperplasia is a feature of many intestinaal nematode infections and is also associated with mast cell hyperplasia, though the two seem independent of each other (Rothwell, 1989). It has been suggested that mucus serves to entrap worms, both incoming larvae and mature worms being expelled. Antibody in mucus may add to the anti parasitic effect (Miller, 1987). Drugs which interfere with mucus function also inhibit worm expulsion (Miller and Huntley, 1982).

IMMUNE UNRESPONSIVENESS OF ANIMAL TO PARASITES

Neonatal unresponsiveness:

A major handicap in the development of vaccines against gastrointestinal nematodes of ruminants is the poor immunological response of lambs and calves. for example, to parasites such as *H. contortus* and various other trichostrongyles. Thereby, animals are at subtantial risk to infection for several months prior to the acquisition of protection which, when acquired, is strong (Lloyd and Soulsby, 1987).

Lambs may not respond effectively to *H. contortus* or *T. colubriformis* untill they reach 3-6 months of age and strong protection may require several more months (Smith & Angus, 1980). A similar unresponsiveness occurs in calves, but this is not so well documented as in sheep. Both parasite and host factors are concerned. For example, whereas young lambs are unable to develop protection against re-infection with most gastrointestinal nematodes, in the case of infection with *Nematodirus battus* lambs can mount a prompt and effective immune response (Lee and Martin, 1976). In addition, lambs can mount an effective immune response to a wide range of bacterial and viral antigens (Lloyd and Soulsby, 1987).

The mechanisms of this immunological unresponsiveness are not fully understood, and various hypotheses on the defect(s) include feed-back inhition by maternally transferred antibody, colostral transfer of soluble antigen or soluble supressor factors and the generation of suppressor cell populations in the neonate. In part, the response has a genetic base and lambs may be segregated into "responders" and non-responders" (Windon et al, 1980). Also, the infection status of the ewe may be important. Thus, immunisation with soluble antigens of 4-week-old lambs against infection with *H. contortus* induced a degree of protective immunity, but only if the lambs had been born of non-infected ewes. This degree of protection was abrogated if the ewes carried an infection with *H. contortus* (Shubber et al, 1984; Lloyd and Soulsby, 1987).

Although there is an early failure of young ruminants to develop an effective immunity to gastrointestinal nematodes, early infection of such animals does not prevent this subsequent development of protective immunity when these animals are re-infected at a later date.

Adult sheep either injected with antigens of *H. contortus* or infected with the parasite have antigen reactive peripheral blood lymphocytes. More than 90% of these cells are cd4+ T cells and approximately 8% are cd8 + T cells (Haig et al, 1989). The immunodominant third stage larval antigens were 15-18, 25-29, 79-80 and>100kD (Haig et al, 1989).

Neonatal responses of lambs to *H. contortus* have shown that peripheral blood lymphocytes of foetal lambs respond well to *H. contortus* antigen, but this responsiveness ceases at birth and remains suppressed untill it reappears (in the absence of any futher stimulation by antigen) 10-12 weeks after birth (Monsell, 1985) Lymphocytes of neonates injected at or shortly after birth with *H. contortus*-antigen fail to respond whereas animals injected at 8-12 weeks of age or when adult do respond (Monsell et al, 1984). The unresponsiveness is associated with colostrum though there is no evidence that passively acquired maternal antibody is responsible (Losson, 1985). The solution to this problem would allow vaccination regimes to be instituted in young animals whereas at present the failure to respond is a severe hindrance to any vaccination scheme.

Periparturient immununosuppression

Immune suppression during pregnancy and lactation is well recognised; it is generalised and non-specific (Smith, 1981), associated with splenomegaly, and with a reduction of T-and -B-cell responses to certain mitogens (Weppner and Coggin, 1980). Immunosuppression appears to be induced by the foetus on the mother and, for example, incubation of lymphocytes from a non-pregnant female with foetal, or neonatal, lymphocytes suppresses the responses and both T-and B-cells (Holt, 1984). The mechanism by which parasites, particularly gastrointestinal nematodes, escape rejection mechanisms during the peripaturient period have yet to be fully elucidated.

However, periparturient immunossuppression is an important component in the epidemiology of gastrointestinal parasitism of domestic livestock. The defective immune response at this time makes an important contribution to the ecology of a parasitic infection. The life cycle of the host and parasite is synchronised to the benefit of the parasite and the increased parasite

activity provides a source of infection for the fully susceptible generation of offspring following pregnancy(Lloyd and Soulsby, 1987).

Genetic aspects of resistance to parasites:

There is increasing evidence that, especially in chronic parasitic infection of ruminants, the genetic make-up of the host influences the outcome of infections (Barger, 1989). Thus, various breeds of cattle and sheep differ in their susceptibility to infection with gastrointestinal nematodes. For example, Scottish blackface sheep are more resistant to *H. contortus* worm-establishment from both primary and secondary infections than Finn Dorset, and increasing susceptibility to infection with *H.contortus* was seen in Red Masai, Blackheaded Persian, Merino, Dorper, Hampshire and Corriedale, both in the field and following experimental infection (Soulsby, 1985). Lambs can be segregated into "responders" and "non-responders" based on immunisation with irradiated *T. colubriformis* larvae and the progeny of "responder" sires and random-bred ewes were more resistant after immunisation than were progeny of "non-responder" sires. Progeny resistance was increased through selection of both sire and dam for responsiveness (Windon et al, 1980).

An association between an ovine lymphocyte antigen (SY1) and the response to vaccination against *T. colubriformis* has been demonstrated. The antigen was present in high frequency on the lymphocytes of responder rams (72.2%) and in lower frequency (21.9%) on the cells of low-responder rams. For ewes, the frequency was 65.7% and 33.5% respectively. A similar association between the SY1 antigen and low faecal egg count was noted in random-bred sheep vaccinated with irradiated larvae and challenged with normal larvae. It has been concluded that the SY1 antigen is likely to be part of the sheep's major histocompatibility complex (MHC) (Outteridge *et al*, 1985).

Futher work with genetic selection is an obvious avenue of investigation. The role of immunity on a herd basis and its implication for parasite control has been examined recently (Anderson &May, 1985). Studies of human parasites (hookworms, schistosomes, filarids) emphasise points well established in the veterinary field, namely that parasite numbers per host are highly aggregated, with only a few hosts harbouring most parasities and most hosts harbouring few parasites. They also recognise that in man the predisposition to heavy infection is probably genetic in origin, and the individuals who harbour few worms are genetically "high responders" and those with heavy burdens, "low responders". Such categories are very similar to the "responders" and "non-responders" recognised in lambs based on immunisation with irradiated *Trichostrongylus colubriformis* larvae.

Naturally acquired herd immunity has important implications on the control of parasitic infections. The variations of this in the animal field contribute significantly to the epidemiology of infection. Mass chemotherapy at a level less than that required to eradicate the parasites can significantly reduce the level of herd immunity, and raise the average worm burden above the levels existing prior to anthelmintics "control" (Anderson and May, 1985).

While the aim of sucessful immunisation programme is the conversion of "non-responders" into "responders", the long term goal may well be to convert the flock to a high responder category by selective breeding, so that the highly aggregated distribution is avoided.

CONCLUSION

Major advances are being made in the understanding of the immune response to parasitic infections and several practical vaccines are now within sight. These rely on the new molecular biological approaches to provide immunising epitopes in usable amounts. How-

ever, other approaches such as the breeding of parasite resistance animals are well within the bounds of possibility in the coming decade.

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