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A Review of Studies on the Immunization against the Pathogenic Protozoan Diseases of Man*

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Abstract

This paper reviews selected studies on the immunization of man and animals against the pathogenic protozoans of man. In most of these diseases (except cutaneous leishmaniasis), there is no practical procedure that can be used to immunize man.

In experimental malaria, both irradiated sporozoites and blood stages have been used successfully to immunize animals. Dead parasites or their soluble antigens are also effective in evoking protective immunity in experimental animals. Some immunity can be passively transferred from the immune mother to her offsprings.

Attenuated or low virulent strains of *Trypanosoma cruzi* can induce protection in animals against more virulent strains. Immunization with dead parasites is less successful.

Mass vaccination programs are now being conducted in certain endemic areas against cutaneous leishmaniasis. The immunizing strain has to be virulent and immunity to challenge only occurs after the healing of the initial ulcer. Immunization with dead parasites is less effective.

In visceral leishmaniasis, humans experimentally infected with strains from wild animals were protected against challenge with infective cultures of human strains. However, in field trial, this cross protection was not demonstrated.

Animals infected with an avirulant strain of *Toxoplasma* are protected against a more virulent strain. Animals can also be protected if they are immunized with dead parasites. In humans, drugs are used mainly to suppress the proliferation of the parasite until the host has acquired sufficient immunity to control the disease.

Live and dead cultures of *Trichomonas vaginalis* are effective in eliciting protection in animals. Sera of infected patients also have protective value when inoculated into mice. Heat-killed cultures when inoculated into vaginal mucosa of infected women was effective in either aborting the infection or alleviating the symptoms of the disease.

Nothing very much is known about protective immunity against amoebiasis and balantidiasis.

Immunization, as discussed in the present paper, refers to the specific protective immunity acquired against a particular organism. This protective immunity may be acquired either actively or passively.

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Actively acquired immunity is produced by the introduction of the live organism (naturally acquired active immunity) or its products (artificially acquired active immunity). The former can be obtained either by allowing the disease to run its course so that on recovery the host becomes immune to challenge (e.g. in human cutaneous leishmaniasis) or the host may build up immunity following a controlled infection. This may be brought about either by the use of drugs (e.g. in human toxoplasmosis) or by depriving the microorganism of some of its essential metabolic requirements (e.g. in experimental rodent malaria) or by the use of attenuated strains of parasites (e.g. in experimental *Trypanosoma cruzi* infection). Artificially acquired active immunity may be produced by the injection of either killed microorganisms (e.g. in experimental toxoplasmosis) or its soluble serum antigens (e.g. in experimental malaria) or the excretion and secretion of live organisms (e.g. in experimental trichomoniasis).

Passive immunity is protection of the host by the introduction of specific antibodies from an actively immunized host. Such protection may be obtained by the injection of specific serum antibodies into a normal host (e.g. in experimental trichomoniasis) or through the placenta from mother to offspring (e.g. as in malaria). Besides prophylactic effects, the immune serum may under certain circumstances be used for therapeutic purposes (e.g. in human trichomoniasis).

As can be seen from the following review, a great deal of information has accumulated from experiments designed to immunize animals against the pathogenic protozoans of man. However, there is as yet no practical procedure which can be used to immunize man himself except for cutaneous leishmaniasis.

This paper is not meant to be an exhaustive review on the subject. However, its purpose is to summarize selected literature in the hope that perhaps some of the experimental designs which had been used so successfully in animals could be considered and adapted for the immunization of man against the same or a related protozoan disease.

Malaria

Most members of the genus *Plasmodium* which infect man and animals are pathogenic. In man, the disease is considered to be the greatest killer of the human race (GARNHAM, 1966). In recent years, the disease has been eradicated or has been brought under control in certain selected areas by the extensive use of insecticides and drugs. However, it is still highly endemic in most areas. In general, the prolonged use of insecticides and drugs for the control of malaria tend to encourage the development of insecticide-resistant mosquitoes and drug-resistant

parasites. Immunization of man against the disease would be the ideal solution, but as yet, there is no practical procedure by which man can be artificially immunized against malaria. Since the unsuccessful attempts by HEIDELBERGER et al. (1946a, b, c, d) to induce protection in man against *Plasmodium vivax* using dead parasites, no serious attempts have been made in recent times.

Most of the work on immunization against malaria parasites were carried out in rats and mice infected with *Plasmodium berghei*. *P. berghei* infection elicits an immunity which controls the course of a primary infection and confers immunity to later homologous challenge. Some investigators obtained evidence that protective antibodies were responsible for acquired immunity to this parasite (DIGGS & OSLER, 1969) while others maintained that cellular immunity may also be involved in this resistance (STECHSCHULTE, 1969).

Active Immunity

(i) Cross protection

JAMES (1931) showed that induced malaria due to *P. vivax* and *P. falciparum* did not stimulate cross protection and even *P. vivax* strains from two different areas would not cross protect. BOYD et al. (1938) and SADUN et al. (1966) also demonstrated no cross protection between strains of *P. falcicarum* from different localities.

CADIGAN and CHAICUMPA (1969) reported that even in a small geographic area (10 mile radius), immunologically distinct strains of *P. falciparum* can be found. They observed that single infection with these strains may confer little or no protection against each other but that multiple reinfection with homologous strains may confer some degree of immunity to some heterologous strains as well. Similar observations were made earlier by BRAY et al. (1962), who found that Africans living in a hyperendemic malaria area responded in a similar way to a strain isolated 240 miles away as with the strain in their home area. Also, MCGREGOR et al. (1963) showed that gamma globulin from West Africa was effective against *P. falciparum* from East Africa.

Recent studies (YOELI et al., 1966; COX & VOLLER, 1966) have shown that mice which have recovered from a low-pathogenic species, *P. chabaudi,* would resist challenge by a pathogenic species, *P. vinckei.* This is perhaps not very surprising because these two species closely resemble each other in morphology and other biological characteristics (GARNHAM, 1966). However, no protection was afforded when the mice were challenged with *P. berghei.*

An attempt to immunize human volunteers against *P. vivax* by initially infecting them with *P. cynomolgi* was unsuccessful (TOBIE et al., 1966).

(ii) Immunization with living parasites

It is generally accepted that full protective immunity to malaria develops slowly and only after repeated exposure (MCGREGOR et al., 1956; MCGREGOR & GILLES, 1960).

In NMRI-mice a patent infection of about 10 to 14 days was necessary for acquisition of good protective immunity while in Swiss mice about 36 days of patent infection was essential (KRETSCHMAR, 1962; JERUSALEM, 1966a, b). Hence, good immunity could be produced in NMRI mice whose infection was controlled by giving the mice a para-aminobenzoic acid (PABA)-deficient diet (KRETSCHMAR, 1962; JACOBI & KRETSCHMAR, 1962). However, in Swiss mice, where a longer patent infection was necessary to produce protective immunity, the mice maintained on the PABA-free diet had to be inoculated with about 500 living parasites per week for 4 weeks. Under such conditions, 81.2 % of mice were immune to challenge (JERUSALEM, 1968). More recent studies (KRETSCHMAR & VOLLER, 1973) have shown that *P. falciparum* infections can be suppressed in owl monkeys *(Aotus)* which were kept on a milk diet.

WEISS & DE GIUSTI (1964, 1966) and WEISS (1968) attenuated a strain of *P. berghei* by passing it alternately in rats and *in vitro*. This strain produced strong immunity in mice when the mice were challenged with virulent strains.

Immunization by use of irradiated blood stages of plasmodia was first reported by CEITHAML and EVANS (1946). They found that irradiated blood stages of *P. gallinaceum* conferred resistance to chickens. Subsequently, CORRADETTI et al. (1966) and WELLDE & SADUN (1967) found that inoculation of mice and rats with irradiated blood forms of *P. berghei* effectively protected them against subsequent challenge. Further studies (WELLDE et al., 1969) showed that the immunity conferred by irradiated blood stages was dose-dependent in regard to both the immunizing and challenge doses. Some immunized mice were resistant to challenge for at least 6 months and these mice developed a reticulocytosis, a slight fall in haematocrit and increased serum gamma globulin. Although these animals could be infected by sporozoite challenges, the resulting infections were rapidly controlled and cleared from circulation.

SADUN et al. (1969) showed that owl monkeys could develop resistance to infection with *P. falciparum* after 4 weekly immunization doses of irradiated blood stages. In these animals, the parasites were

inhibited from multiplying and this permitted the animals to survive lethal challenges with non-irradiated parasites. No immunity was detected in monkeys which received one dose of irradiated parasites while those that received three weekly doses were not highly resistant to challenge.

Irradiated sporozoites of *P. gallinaceum* would effectively protect fowls against a challenge with infective homologous sporozoites but not with trophozoites (RICHARDS, 1966). Similar results were also obtained with *P. berghei* in rats (NUSSENZWEIG et al., 1969), which showed that, while a single dose of irradiated sporozoites would confer partial protection, 100% protection could be obtained by repeated injections or by using larger doses. A dose of 7.5×10^4 irradiated sporozoites is sufficient, but when repeated, would completely prevent the development of patent infections when challenged. Also there is considerable loss of infectivity when sporozoites were incubated *in vitro* with sera of immunized animals. Sporozoites of other rodent malaria parasites, *P. vinckei* and *P. chabaudi,* are also affected by sera of immunized animals (VANDERBERG et al., 1969) but only at lower serum dilutions than homologous sporozoites. However, sporozoites of avian malaria *(P. gallinaceum)* and simian malaria *(P. cynomolgi)* are not affected.

(iii) Immunization with dead parasites and soluble antigens

Protective immunity to homologous challenge has been shown in rhesus monkeys after they were inoculated with killed *P. knowlesi* schizonts in Freund's complete adjuvant (FREUND et al., 1945, 1948; TARGETT & FULTON, 1965). However, if Freund's incomplete adjuvant was used with killed parasites, the resultant immunity is only variant-specific (BROWN et al., 1968). Animals which were given killed parasites and complete adjuvant developed an infection when challenged after prolonged incubation period. This infection lasted for not more than 2 weeks, after which the animals eliminated the infections and appeared to be completely protected from further challenges.

However, dead schizonts of *P. falciparum* with Freund's adjuvant only confer a slight immunity to *Aotus* monkeys (VOLLER & RICHARDS, 1968). The challenged animals showed a longer prepatent period and eventually died from the infection.

ZUCKERMAN et al. (1967) immunized weanling rats with a pressure-disintegrated homogenate of *P. berghei* without adjuvants. They found that there was prolonged prepatency, reduced duration of patency and peak parasitemia and lower mortality in challenged animals.

Sporozoites of *P. berghei* inactivated by heat or by freezing and thawing when inoculated repeatedly into mice did elicit good im-

munity in mice. Most of the immunized mice were resistant to intraperitoneal challenge of 2,000 sporozoites (ALGER et al., 1972) and 3 of 9 mice inoculated with protein material of uninfected salivary gland were also resistant to infection by sporozoites.

The route of inoculation of antigen was found to be very important (JERUSALEM & ELING, 1969). Intravenous injection was far superior to intraperitoneal and intramuscular inoculations. It not only had the largest number of surviving mice on challenge but also the greatest degree of antiparasitic immunity. Immunization of animals can also be achieved by the inoculation of soluble antigens recovered from the sera of infected animals.

COX (1966) found that soluble antigens from the sera of heavily infected monkeys produced a relatively mild anemia when inoculated into rats. Subsequently these rats become very resistant to blood-induced *P. berghei.* Similarly, all 15 chickens immunized with soluble antigen derived from sera of previously infected fowl survived a challenge of blood stages of *P. gallinaceum,* while all 20 control chickens died from the infection in 6 to 12 days (TODOROVIC et al., 1968).

Passive Immunity

Rats which were given a series of injections of hyperimmune serum against *P. berghei* had longer prepatent periods when they were challenged (FABIANI & FULCHIRON, 1953; FABIANI & ORFILA, 1956). Similar protection was conferred on mice which had received hyperimmune rat serum (MARTIN et al., 1966). However, the sera of infected mice were nonprotective (FABIANI, 1954).

Female rats hyperimmunized to *P. berghei* during gestation gave birth to youngs which were more resistant to infection (BRUCE-CHWATT, 1954; BRUCE-CHWATT & GIBSON, 1956). According to these workers this protection was transferred chiefly through the milk. The more immune the mother rat, the more effective is this sort of passive transfer (BRUCE-CHWATT, 1963; DEMINA, 1958; ISFAN & IANCO, 1964). TERRY (1956) showed that new-born rats which were fed immune rat serum were more resistant to challenge. This ability to absorb antibody through the gut wall ceased soon after the rats were weaned. However, GAIL et al. (1967) showed that this protection in mice "... is not directed against the parasite, but enables them (the mice) to survive the severe infection longer than equally young mice from normal parents". Also, because antibodies have not been demonstrated conclusively to be present in the milk, some workers (KRETSCHMAR & VOLLER, 1973) felt that other factors might be responsible for the protection.

It is generally considered that passive immunity does not protect young children from malaria, although it may alter the severity of the disease. COHEN & MCGREGOR (1963) were successful in the passive transfer of immunity in man. They showed that gamma G immunoglobulin in the blood of immune persons was capable of dramatically reducing parasitemia and that this could be transmitted transplacentally from immune mother to offspring (MCGREGOR, 1964).

It was also shown that immune gamma globulin from West African natives had prophylactic and suppressive effect in splenectomized chimpanzees infected with blood induced *P. falciparum* from the same area. However, the gamma globulin from West Africa had no marked effect in chimpanzees infected with a drug resistant strain from South-east Asia (SADUN et al., 1966).

American Trypanosomiasis

American trypanosomiasis or Chagas' disease is caused by *Trypanosoma cruzi*. According to WHO report (WHO, 1962), the number of infected individuals was estimated to be at least 7 million people. *T. cruzi* has a wide range of mammalian hosts (WOO & SOLTYS, 1970).

Our knowledge of immunity in Chagas' disease is still incomplete and we still do not know whether the blood stages or tissue stages initiate the immune response. In mice, the blood stages are important for in these animals the blood phase is prolonged while in man, trypanosomes spend only a short time in the blood, and are mainly in the form of tissue stages. In view of this, there is considerable controversy regarding the mechanism of the immune response in Chagas' disease. Some workers believe that cellular factors are almost exclusively responsible for the development of immunity in man (PIZZI, 1961), while others are of the opinion that humoral antibodies are of fundamental importance in the mechanism of immunity (MUNIZ, 1946).

COLLIER (1931) has shown that resistance to reinfection depends upon the persistence of a latent infection in the host. Cross immunity between strains of *T. cruzi* observed by BRUMPT (1913) was confirmed by many other workers (see GOBLE, 1970).

Active Immunity

(i) Immunization with living trypanosomes

Observations by early workers that infection of mice with a strain of low virulence led to cross-resistance to challenge with a virulent

strain suggested a search for an effective vaccine. COLLIER (1931) attenuated a strain of *T. cruzi* by subjecting it to trypaflavine and used it to produce mild infections in mice which were thereafter resistant to challenge with a virulent strain.

Further studies with strains attenuated by long passage in culture (PIZZI & PRAGER, 1952; MENEZES, 1968) or with strains of naturally low virulence (HAUSCHKA et al., 1950; NORMAN & KAGAN, 1969) have shown that these strains were able to induce protective immunity to challenge with highly virulent strains.

KAGAN & NORMAN (1961) showed that almost all mice were protected against a virulent strain of *T. cruzi* if they were challenged from 28 days to a year after they had been infected with culture forms of an avirulent strain. More recently, SEAH and MARSDEN (1969) showed that 13 of 15 mice infected with an avirulent strain for 14 days survived at least 3 months after being challenged. All controls and those that were challenged 2 and 7 days after infection with an attenuated strain died within 14 days. The highest values were observed when the interval between vaccination and challenge was 3 weeks or more.

FERNANDES et al. (1966) observed that the addition of actinomycin D to cultures of *T. cruzi* inhibits multiplication but preserves motility. These flagellates were living, but non-infectious. When these were given to mice in doses of 3×10^8 intraperitoneally once per week for 4 weeks, all the 18 immunized animals had a low grade blood infection when challenged with a virulent strain. They were alive at 90 days, while all controls were dead in 13 days.

BROWNING et al. (1946) found that infected mice which had been treated with phenanthridinium compounds were refractory to subsequent inoculations with the homologous strain, even many months after the primary infection. HAUSCHKA et al. (1950), after treating mice infected with virulent strains with Bayer 7602, observed that these animals were highly resistant to challenge with either homologous or heterologous virulent strains.

(ii) Immunization with dead trypanosomes

Early workers were unsuccessful in immunizing animals with killed cultures (MUNIZ et al., 1946; HAUSCHKA et al., 1950; KAGAN & NORMAN, 1961); however, more recent workers were more successful. It has been found that the physical methods such as sonication or rapid freezing and thawing are more effective than chemical means in preserving protective antigens. JOHNSON et al. (1963) were able to demonstrate immunity in mice which were inoculated with *T. cruzi* killed by freezing and thawing. Saponin was found to be the most effective ad-

juvant used. Sterile immunity did not result on challenge; there was only a decrease in parasitemia and prolonged survival times. SENECA et al. (1966) showed that a lipopolysaccharide extracted from culture forms of *T. cruzi* when inoculated into mice afforded good protection for 40 days provided that mice received at least 7 doses. If mice were given 16 doses and challenged 4 weeks after the last dose, the immunized mice lived for more than 140 days.

African Trypanosomiases

Immunization against African trypanosomiases has been reviewed by SOLTYS (1973). The decision to omit this section in the present review was taken because most of the work was conducted with species pathogenic to domestic animals and hence would be more appropriate in the other review (SOLTYS, 1973).

Leishmaniasis

Cutaneous Leishmaniasis

The causative agent of cutaneous leishmaniasis (oriental sore) is *Leishmania tropica.* For years, it has been the custom of people in endemic areas to inoculate their children on selected parts of their body with materials taken from ulcers caused by the organisms (WENYON, 1911; MANSON, 1914). This was to prevent the development of scars on the face or hands due to a later natural infection.

Active Immunity

(i) Immunization with living parasites

BERBERIAN (1939) vaccinated volunteers with cultures of *L. tropica* and found that a single sore, either experimentally or naturally acquired, conferred definite immunity to a challenge of infective culture forms. Subsequent studies (SENEKJI & BEATTIE, 1941) showed that ulcers were produced in all 227 persons who were inoculated with cultures of *L. tropica* and that immunity to a challenge infection was only demonstrated in persons whose ulcers healed at the time of challenge. The healing of ulcers required at least 240 days (BERBERIAN, 1944) and that immunity to challenge was not developed until the initiation of the healing of the ulcer.

In central Asia, cutaneous leishmaniasis occurs in two clinical forms. In such a situation, the proportion of non-immune individuals who develop an ulcer on vaccination is dependent on the strain used. This may vary from 70 % with the dry strain *(L. tropica major)* to almost a 100 % with the wet strain *(L. tropica minor)*. Individuals who have recovered from *L. tropica major* will be protected against *L. tropica minor* as with homologous strains, but infections with *L. tropica minor* do not cross protect (KOJEVNIKOV, 1945; KOZEVNIKOV, 1963).

The virulence of the challenge strain was also shown to be important in the assessment of immunity on challenge. KELLINA (1966) found that a low virulence strain produced only delayed-type local skin reaction in immune individuals similar to that produced by the injection of killed promastigotes. However, with a virulent strain, nodular formation occurred and parasites were often demonstrated. Necrosis with ulcer formation occurred in some individuals.

Recent mass vaccination programs conducted by the Russian workers (SEREBRYAKOV et al., 1968; SERGIEV et al., 1970) against *L. tropica major* showed the virulence of the immunizing strain was important in the establishment of immunity to challenge. In the latter study, there was only one apparent failure to protect in 8,195 persons vaccinated with a virulent strain. However, 128 out of 1,280 vaccinated persons were not protected because they were vaccinated with a low virulence strain. More recent studies (NAGGAN et al., 1972) have confirmed this. A group of 81 adults vaccinated with a virulent strain of *L. tropica* were protected from natural infections while 30 % of the control group became infected during the same period. It was also found that the leishmania prepared from the virulent strain gave significantly better results than leishmania prepared from another strain which had lost its infectivity for man.

Reinfection after recovery

There have been reports of natural reinfections in patients who had recovered from the infection. Some of these people had initially acquired the infection at one place and had later moved to another endemic area. For example, KOJEVNIKOV (1945), in going through the case histories of 1894 patients in Ashkhabad, had found 86 records of reinfection. Most of these reinfections had occurred in patients who had moved from one endemic area to another. RAHIM and TATAR (1966) had also reported reinfections in two patients who had initially acquired their first infection elsewhere.

Some reinfections might have been due to inhibition of the immunological mechanisms of the host due to drugs. GUIRGES (1971)

felt that 3 of the 6 reinfections that he studied might have been as a result of the therapeutic use of corticosteriods or corticotrophin.

LATYSHEV and KRYUKOVA (1953) speculated that immunity might not be lifelong because they achieved a reinfection $7^1/_2$ years after recovery from the initial lesion.

Other speculations were that an unusually large dose of a highly virulent strain might have been inoculated by the sandfly or there was a breakdown of cell-mediated immunity so that the parasitized histiocytes were not destroyed (GUIRGES, 1971).

(ii) Cross immunity

CONVIT (1958) demonstrated that on recovery of a *L. brazilensis* infection, protection against the homologous strains was good while no such protection was afforded when challenged with a heterologous strain *(L. pifanoi).*

As *L. tropica major* will protect against *L. tropica minor,* but not vice versa (KOZEVNIKOV, 1963), the zoonotic strain of *L. tropica major* has been used for vaccination in man. ADLER & GUNDERS (1964) found complete resistance to challenge with *L. mexicana* in two human volunteers who had been infected experimentally 2 to 3 years before with *L. tropica.*

Although *L. mexicana* has been shown to be antigenically distinct from a Panamanian strain of *L. brazilensis* (LAINSON & SHAW, 1966), it will protect against the latter strain but no protection was shown against a metastasizing strain of *L. braziliensis* in monkeys (LAINSON & BRAY, 1966).

(iii) Immunization with dead parasites

Although most workers believed that protective immunity can only be achieved with living organisms, there have been several reports of successful vaccinations with dead leptomonads. PESSOA (1941), PESSOA & PESTANA (1941) and DE SAMPAIO (1951) reported on the successful induction of protective immunity after several injections of killed parasites. Also, COUTINHO (1954, 1955) demonstrated that partial protection could be induced in guinea pigs against *L. enriettii* after the injection of three or four doses of killed parasites. However, LAINSON & BRAY (1966) found that *L. mexicana* killed in formal-saline did not induce protective immunity when inoculated into hamsters and mice.

More recent studies (PRESTON & DUMONDE, 1971) showed that the ribosomal antigen of *L. enriettii* inoculated with Freund's incomplete

adjuvant into guinea pigs elicited strong resistance to challenge while no protective immunity could be induced by immunization with mitochondria, nuclei or flagella. Although circulating antibodies are produced in immune guinea pigs, these sera have no protective value (KRETSCHMAR, 1965).

Passive Immunity

ADLER & NELKEN (1965) were unable to transfer the delayed hypersensitivity skin reaction from a hypersensitive person to four human recipients. Two were injected with washed leucocytes and two were given whole blood. Similarly, BRAY and LAINSON (1965) were unsuccessful in similar passive transfer on humans, monkeys, rabbits and guinea pigs. However, none of the recipients were tested for the transfer of protective immunity.

Visceral Leishmaniasis

Visceral leishmaniasis or kala-azar is caused by *L. donovani* and it is often considered a fatal disease if untreated. However, spontaneous recoveries have been recorded and according to some workers, this may be as high as 25 % (NAPIER, 1946, in India; FRAGA DE AZEVEDO, 1960, in Portugal). Also an atypical and asymptomatic kala-azar has been detected in certain individuals living in endemic areas. This chronic, almost inapparent form of the disease is not fatal.

Since reinfection in successfully treated patients have not been reported (SEN-GUPTA, 1948), it was suggested that immunity is long-lasting. According to MANSON-BAHR (1961) immunity conferred by a natural infection lasts for at least 10 years.

Active Immunity

(i) Immunization with living parasites

The experimental proof of human resistance to reinfection with *L. donovani* was not forthcoming until the reports of MANSON-BAHR (1959, 1961).

He showed that inoculation of cultures of human kala-azar strain in man would initially produce a small local nodule and later the parasites spread to the visceral. The inoculation of cultures derived from gerbil or ground squirrel would cause similar nodules in man, but the parasite did not spread to the visceral. Six to eight weeks later, the

nodules subsided and the volunteers became immune to challenge infections in the skin with cultures of the human strain of kala-azar.

Also, patients cured of kala-azar and similarly injected with cultures of East African, Mediterranean, Indian, or rodent strains of *L. donovani* showed only temporary nodules and repeated culture failed to demonstrate the presence of parasites in these people.

As a result, large scale immunization of people living in endemic areas using ground squirrel strains of *L. donovani* was conducted in Kenya (MANSON-BAHR & SOUTHGATE, 1964). However, cross-protection was not demonstrated in the field trial.

(ii) Cross immunity

MANSON-BAHR (1961) injected subcutaneously into the treated patients (cured of kala-azar), cultures of several strains of *L. tropica* ("moist"- and "dry"-type parasites) and demonstrated the lack of cross immunity between *L. donovani* and *L. tropica.* Earlier studies (PATTON, 1922) have shown that recovery from cutaneous leishmaniasis does not protect against kala-azar.

Passive Immunity

OTT (1964) demonstrated that mice which received sera from immune guinea pigs had a lower peak infection and an earlier fall in numbers of parasites in the liver compared to mice which received normal guinea pig serum or no serum. BOYSIA (1967), using spleen and lymph node cells from sensitized guinea pigs, was successful in transferring the delayed hypersensitivity skin reaction. However, he did not test the recipient animals for the transfer of protection.

In vitro studies (MILLER & TWOHY, 1969) showed that macrophages from infected mice were resistant to intracellular forms compared to macrophages from normal mice. However, serum from infected mice when added to cultures did not significantly change neither the rate of parasite multiplication in macrophages from normal mice nor the rate of destruction of parasites from infected mice.

Toxoplasmosis

Toxoplasma has been isolated from a wide range of animals and experimental studies have shown that no mammal or bird is fully resistant to experimental infection. The course of infection and the development of immunity are dependent on the strain of parasite, the

susceptibility and age of the host, the route of infection and the size of the inoculum.

Animals that survive an infection generally develop immunity (premunity) with cysts persisting for a long time. FRENKEL (1953) believed that acquired immunity is dependent on many factors of which humoral antibody is only one. According to HULDT (1966) circulating antibody does not play an important role in protection and the resistance was probably due to cellular immunity. Recent *in vivo* and *in vitro* studies have shown that the parasite is sensitive to interferon and interferon inducers which would indicate that non-specific cell mediated resistance should also be considered (REMINGTON, 1970).

Active Immunity

(i) Immunization with living parasites

WEINMAN (1943) showed that mice infected with an avirulent strain survived a lethal challenge dose while EICHENWALD (1949) demonstrated good immunity in mice after they had been infected with an avirulent strain for 3 weeks. FRENKEL (1956) was able to detect immunity in mice infected with an avirulent strain after 1 week and confirmed that this was well established in 3 weeks.

In mice, the avirulent Beverley strain of toxoplasma protects against the virulent RH strain (STAHL & AKAO, 1964). This protection reaches its peak after 4 weeks. Mice protected in this way still harbour the parasite in the cystic stage in the brain and other tissues 3 to 8 weeks after challenge although they disappear from the peritoneal cavity in 7 days. Control mice died in about 10 days (NAKAYAMA, 1964, 1966). MATSUBAYASHI and AKAO (1966) showed that the cyst protects the toxoplasms against the protective antibodies. They labelled anti-toxoplasma serum with ferritin and showed that it is located on the surface of free toxoplasms, while in the cysts, the ferritin did not reach the parasite.

Similarly, immunity was demonstrated in guinea pigs infected with avirulent strains. CUTCHINS & WARREN (1956) showed that these animals resisted a lethal challenge 1 month after immunization, while later studies showed that this resistance to challenge varied from 2 to 8 months (FOSTER & MCCULLOCH, 1968; HULDT, 1963).

Guinea pigs infected with a low virulent strain (originally isolated from human lymph nodes) survived when they were challenged with 1,000 parasites of a highly virulent strain. However, when they were challenged with 10,000 parasites, the guinea pigs died as did the con-

trols (PIEKARSKI, 1966). More recent studies on the use of avirulent strains to evoke immunity in mice and guinea pigs were conducted by KRAHENBUHL et al. (1971). They found that on inoculation of tissue culture-grown trophozoites of an avirulent strain, the host developed immunity to a lethal challenge with a virulent strain within about 2 weeks.

Pigs that had survived an infection developed a strong immunity against challenge with homologous and heterologous strains. The initial infection need not be acute to produce a strong immunity (FOLKERS, 1962).

Trophozoites are susceptible to various drugs and hence drugs are used in human infections mainly to suppress the proliferation of the parasite until the host has acquired sufficient immunity to control the disease (FELDMAN, 1968; FRENKEL, 1971).

(ii) Immunization with dead parasites

Guinea pigs can be immunized with dead organisms against the parasite (CUTCHINS & WARREN, 1956). The animals did not die nor were they severely ill as the controls when they were challenged with the homologous strain. Further work (RODRIGUEZ, 1957) showed that guinea pigs can be immunized with a single injection of formalized organisms (isolated from a pigeon) plus adjuvant of lanolin and petrolatum. These animals were protected when challenged with 10 million organisms 16 days later.

WILDFUHR (1957) also showed that rabbits immunized with 16 inoculations of inactivated organisms were protected against lethal challenge doses while hamsters were not thus protected.

Trichomoniasis

Most trichomonads are nonpathogenic, but a few are important pathogens. None of the cecal trichomonads have been shown to be pathogenic and hence will not be discussed in this review.

Trichomonas gallinae is a parasite of the upper digestive tract of birds. The infection may spread to the liver where a large part of the organ may be replaced by the parasite. Bovine trichomoniasis is a genital disease caused by *T. foetus*. The disease can cause abortion in cattle. In man, genital trichomoniasis is caused by *T. vaginalis*. It is worldwide in distribution. The disease is usually asymptomatic in the male. *T. vaginalis* in the female is characterized by leukorrhea and vaginal and vulvar pruritis.

Active Immunity

(i) Immunization with living parasites

STABLER (1951, 1954) has shown that low virulent strains of *T. gallinae* which cause no apparent damage to birds can protect them against more virulent strains.

Heifers can be protected against genital infections due to *T. foetus* if they are given a series of 16 intramuscular or intravenous injections of living parasites over a period of 3 months. However, 6 intramuscular injections over a 3-week period do not confer any protection (MORGAN, 1947).

Intramuscular injection of cultures of living *T. vaginalis* into one hind leg of mice (abscesses at the site of inoculation), protected 80 to 100% of these mice from developing similar abscesses in the other hind leg on challenge (KELLY & SCHNITZER, 1952). There appeared to be good cross immunity between *T. vaginalis* and *T. gallinae* in mice. In mice immunized by intramuscular injections of living *T. vaginalis,* the parasites of a homologous reinfection survived less than 8 hours in the tissues (SCHNITZER & KELLY, 1953). Subsequent work (KELLY et al., 1954) showed that vaccinated mice by intramuscular route were almost 100% resistant to intraperitoneal challenge of *T. vaginalis,* while resistance to subcutaneous challenge was less striking. Similarly, good protection against intramuscular challenge was achieved in mice which had been immunized by intraperitoneal route. Immunization by subcutaneous injections of parasites did not confer any immunity to subsequent challenge via subcutaneous or intraperitoneal routes. However, these mice had good protection against intramuscular challenge for 6 weeks.

(ii) Immunization with killed parasites and their products

Formalin-killed parasites from the urogenital tract protected mice against subsequent intraperitoneal challenge (NAKABAYASHI, 1952). Similar results were obtained by BABA (1957, 1958), who used heat-killed *T. vaginalis, T. gallinae* and *T. foetus.* The dead trichomonads were inoculated intraperitoneally and challenge infection was by intramuscular injection. Effective protection was most pronounced when the challenge was with homologous strain, but there was also a certain amount of protection against heterologous species.

WARREN et al. (1961) inoculated subcutaneously into mice "physiologically derived antigen" (substances secreted or excreted by *T. gallinae in vitro* at 37 °C) and showed that these antigens stimulated the

production of antibodies which protected the mice against challenge by living flagellates.

ABUREL et al. (1963) reported success in treating 100 women with urogenital trichomoniasis. All these patients had drug resistant infections. Forty per cent of the women lost the infection after heat-killed cultured trichomonads were inoculated in increasing dosages into their vaginal mucosa. A further 49 % of the women were alleviated of all symptoms of the disease.

Passive Immunity

NIGESEN (1966) tested 195 human sera for protective effects against intraperitoneal challenge in mice with cultured *T. vaginalis,* 111 sera came from infected patients, 67 sera from parasite free individuals, while 12 sera came from individuals whose sexual partners suffered from trichomoniasis. Thirty minutes after the intraperitoneal inoculation of human sera, the mice were challenged with different antigenic strains of trichomonads. Sera from the 67 non-infected individuals did not protect the mice against intraperitoneal challenge while sera from infected persons did. Also, sera from individuals whose sexual partners suffered from trichomoniasis, had similar protective effects as those from infected persons. The protective effects of sera from infected persons diminished completely six months after treatment.

ABUREL et al. (1963) injected hyperimmunized sera into several areas of the cervix, uteri and vagina of 3 patients whose infection was refractory to drugs and vaccination. One lost the infection while the other two showed great improvement.

Amoebiasis

Different species of animals vary in their natural resistance to *Entamoeba histolytica* infection. Kittens are susceptible while rats and monkeys are relatively resistant to tissue invasion. Since only a small percentage of human beings suffer from clinical amoebiasis, this would indicate that man has a considerable degree of natural resistance to invasion by the parasite. Individual resistance appears to be the chief factor in determining clinical amoebiasis. However, the virulence of the strain, the size and frequency of the infecting doses undoubtedly contribute to overcome the natural resistance.

Nothing is known about acquired immunity to amoebiasis in man (SHAFFER et al., 1965). There is little evidence to show the development of any resistance to reinfection in man. However, studies in dogs by

SWARTZWELDER and AVANT (1952) demonstrated that, despite repeated attempts to reestablish infections in animals whose initial infection had been terminated, only 17 per cent could be reinfected. The resistance to infection was active against both homologous and heterologous strains of *E. histolytica.* The tested duration of immunity to reinfection ranged from two and one-half months to nine and one-half months.

Balantidiasis

The disease is caused by *Balantidium coli,* which is a comparatively rare parasite in man. Man appears to be an incidental and highly resistant host because attempts to infect him experimentally with trophozoites or cysts from pigs, monkeys and man have not been successful (YOUNG, 1950). If *B. coli* does get established in man, it usually becomes a tissue invader but in some individuals, it may become a commensal. It may also cause diarrhea or dysentery.

As in amoebiasis, nothing very much is known about acquired immunity to *Balantidium* infections.

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Zusammenfassung

Die Arbeit gibt eine Übersicht ausgewählter Untersuchungen der Immunisierung von Mensch und Tieren gegen menschen-pathogene Protozoen. Für die meisten der betreffenden Krankheiten, Hautleishmaniasen ausgenommen, gibt es zur Zeit keine praktisch anwendbare Immunisierungsmethode beim Menschen.

Bei experimenteller Malaria wurden mit Erfolg bestrahlte Sporozoiten und Blutformen angewandt, um Tiere zu immunisieren. Abgetötete Parasiten und ihre löslichen Antigene erwiesen sich bei der Immunisierung von Laboratoriumstieren ebenfalls als wirksam. Eine gewisse passive Immunität kann von der immunen Mutter auf ihre Nachkommen übertragen werden.

Stämme von *Trypanosoma cruzi,* von erniedrigter oder schwacher Virulenz, vermögen Tiere gegenüber stärker virulenten Stämmen zu schützen. Abgetötete Parasiten erwiesen sich zur Immunisierung als weniger wirksam.

Neuerdings werden in gewissen endemischen Gebieten Massenimpfungskampagnen zum Schutz gegen Hautleishmaniase durchgeführt. Die dafür benützten Leishmanien müssen virulent sein; eine Immunität tritt erst nach der Heilung der Primärgeschwüre auf. Eine Immunisierung mit abgetöteten Parasiten erwies sich als schwieriger.

Menschen, welche experimentell mit Stämmen visceraler Leishmaniase aus freilebenden Tieren infiziert wurden, erwiesen sich als gegen infektiöse Kulturen menschenpathogener Stämme geschützt. Dieser Schutz ließ sich jedoch in Feldversuchen nicht bestätigen.

Tiere, welche mit einem avirulenten *Toxoplasma*-Stamm infiziert sind, sind gegen virulentere Stämme dieses Parasiten geschützt. Tiere lassen sich auch mit abgetöteten Parasiten immunisieren. Beim Menschen werden Pharmaka vor allem dafür verwendet, die Vermehrung des Parasiten so lange zu unterdrücken, bis der Wirt einen ausreichenden Immunitätsgrad entwickelt hat, um die Krankheit selbst zu überwinden.

Bei *Trichomonas vaginalis* erwiesen sich lebende wie abgetötete Kulturen als zum Schutz von Tieren wirksam. Ebenso sind Seren infizierter Patienten nach Inokulation bei Mäusen wirksam. Die Inokulation hitzeabgetöteten Kulturmaterials in vaginale Mucosa erkrankter Frauen führt zu einem Abbruch der Infektion oder mindestens zu einer Milderung der Krankheitssymptome.

In Zusammenhang mit Amoebiase und Balantidiase ist zur Immunität sozusagen nichts bekannt.

Résumé

Cette publication passe en revue une sélection de travaux portant sur l'immunisation de l'homme et des animaux contre les protozoaires pathogènes de l'homme. Pour la plupart de ces maladies (excepté la leishmaniose cutanée) il n'y a pas de procédé pratique pouvant être utilisé pour l'immunisation de l'homme.

Dans la malaria expérimentale, des sporozoites et des formes érythrocytaires irradiés ont été utilisés avec succès pour immuniser des animaux. Les parasites tués ou leurs antigènes solubles induisent également une protection immunitaire chez les animaux d'expérience. Un certain degré d'immunité peut être transféré passivement d'une mère immune à ses nouveaux nés.

Des souches atténuées ou de faible virulence de *Trypanosoma cruzi* peuvent induire chez l'animal une protection contre des souches plus virulentes. L'immunisation avec des parasites tués est moins efficace.

Des programmes de vaccination de masse contre la leishmaniose cutanée sont maintenant en cours de réalisation dans certaines régions endémiques. Les souches utilisées pour immunisation doivent être virulentes et l'immunité n'apparaît qu'après la guérison de l'ulcère vaccinal. L'immunisation avec des parasites tués est moins efficace.

Dans la leishmaniose viscérale, des hommes infectés expérimentalement avec des souches animales n'ont pas présenté de réactions pathologiques après l'inoculation de souches humaines de culture. Cependant, cette protection croisée n'a pas été démontrée sur le terrain.

Les animaux infectés avec une souche avirulente de *Toxoplasma* sont protégés contre une souche plus virulente. Les animaux peuvent également être protégés s'ils sont immunisés avec des parasites tués. Chez l'homme, des médicaments sont souvent utilisés principalement pour stopper la prolifération du para-

site jusqu'à ce que l'hôte ait acquis un degré suffisant d'immunité pour supporter son infection.

Des cultures vivantes ou tuées de *Trichomonas vaginalis* induisent une protection chez les animaux. Les sérums de sujets infectés présentent aussi un pouvoir protecteur quand ils sont inoculés à une souris. Des cultures tuées par la chaleur et inoculées dans la muqueuse vaginale de femmes infectées ont pu faire avorter l'infection ou atténuer les symptômes de la maladie.

Peu de choses sont connues sur les phénomènes de protection immunitaire dans l'ambiase et la balantidiose.