

# Immunology of Leishmaniasis\*

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*Knowledge of the immunological basis of the leishmaniasis and of the host's response is fragmentary and largely pragmatic. This paper reviews certain conceptual and clinical aspects of the immunology of these diseases. Consideration is given to man's natural resistance and his ability to acquire resistance from natural infections and from vaccination. The age-distribution of infection in different populations is discussed in relation to the effects that interaction between the parasite and its intermediate host may have on its infection characteristics and virulence.*

*Studies in the USSR of differences in virulence among 30 human strains and 39 rodent strains are reported. The rodent strains showed a broader range of virulence than did the human isolates. Serological tests for determining species relationships among the leishmaniasis are generally nonspecific, but work concerned with the development of the antiserum-culture test is reviewed. Species identification and the recognition of new forms, perhaps with different infection characteristics, is, nevertheless, of the utmost importance in the prevention and treatment of the disease.*

*The review concludes with a discussion of functional immunity and hypotheses of the immune process in leishmaniasis.*

## INTRODUCTION

Immunology is fundamentally a measure of the living interaction between parasite and host. This interaction, carefully measured and compared in different *Leishmania*-host combinations, has been our best clue to the biological uniqueness of the parasite, its genetic identity and constancy, and its speciation and evolution.

The key problem for a study of the host's immunological response is to separate invasiveness, pathogenicity, and resistance into their intrinsic host and parasite components. A number of species and strains of *Leishmania* undoubtedly exist. Among the hosts, there are differences in individual as well as in species response to infection. It is often difficult or impossible, therefore, to distinguish between innate virulence of the invader and immunological failure of the host. Yet we characterize *Leishmania* parasites by host susceptibility, resistance,

or pathology when it is observed repeatedly in man or in experimental animals infected in a standardized fashion. In unusual cases, such as in leishmaniasis tegumentaria diffusa, the response can tell us much about the host as well.

Immunology includes the practical application of serological and skin testing procedures in diagnosis and in studies of disease prevalence in addition to its usefulness in distinguishing between *Leishmania* species. Ideally, cultures of unknown *Leishmania* tested against known antisera should enable us to identify the parasite and determine its antigen array and reaction limits; known parasite strains should similarly be useful in identifying test sera. The realization of this goal with *Leishmania* is only in the experimental stage. Most tests are nonspecific or group-specific, permitting detection of antigens shared by related organisms. They do not distinguish antigens confined to a single species, as group reactions mask specific ones. The extreme morphological similarity among related flagellates is paralleled by their many shared antigenic components.

One diagnostic technique often used is the delayed-reaction skin test, the Montenegro (leishmanin) reaction. It is not highly specific and does not

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distinguish current from past infection. Application of the test over large areas, however, may enable us to deduce migration pathways of leishmaniasis or at least past leishmanial foci. It is also useful for the diagnosis of *L. brasiliensis*, especially when parasites are often scarce or difficult to demonstrate, as in *espundia*.

Prophylaxis against cutaneous leishmaniasis is a managed infection involving inoculation of a living culture. Site, duration, and intensity of infection are carefully controlled in this induced disease. Prophylactic infection has been best developed in the Soviet Union and its use protects thousands of workers in endemic areas, persons who otherwise would be disabled or seriously afflicted by disfiguring sores and painful long-lasting ulcers.

Serological tests demonstrate serum antibody, not immunity, in contrast to the prophylactic sore, which stimulates full protection. As with many tissue infections, serological titre does not necessarily measure the host's specific defensive capacity. The two, in fact, may show an inverse relationship. Although serum antibodies are readily detected by the complement fixation test (CFT) in visceral leishmaniasis, host protection does not normally develop. In cutaneous leishmaniasis, on the other hand, no CFT response is obtained, but total immunity usually follows initial infection. This does not rule out an antibody basis for the host's defensive mechanism. It implies rather that such antibodies are cellular and locally concentrated, presumably in the skin or around the infected phagocytes and in some way related to local infiltration by lymphocytes and plasma cells. Antibodies released into the blood are not effective in aiding this localized immune response. The particular pathological characteristics of the various leishmaniae are intimately related to the degree of localization of the host response and the containment of infected macrophages. A reliable immunological test capable of distinguishing cell-bound antibodies and specific tissue-active protective processes is needed. Perhaps the passive cutaneous anaphylaxis (PCA) test could be used. *In vitro* tests might be developed to measure attraction of specifically activated lymphocytes towards infected rather than non-infected macrophages.

To emphasize poorly understood and little-known aspects of leishmaniasis, we can restate some of the host-parasite characteristics of the disease:

(1) It is almost exclusively a disease of the host macrophage (histiocyte) system.

(2) When infected macrophages remain in the dermis, an island of infected cells forms. Eventually this induces a strong host cell reaction of proliferating infected macrophages, plasma cells, and lymphocytes that produces a lesion. Extension of the lesion, ensuing tissue destruction, and local spread of infection cause the development and characteristic ulceration of cutaneous leishmaniasis.

(3) In visceral leishmaniasis, a dermal lesion may develop initially, but this disease is characterized by the escape of infected cells from the dermis into the major lymphocytopoietic and macrophage-filtering centres, that is, into the spleen, the liver, the bone marrow, and the lymph glands. A massive generalized lymphocytogenesis in these organs leads to extreme splenomegaly and hepatomegaly. Accompanying this is an exceedingly heavy hypergammaglobulinaemia, an outpouring of largely nonspecific complement-fixing antibodies into the serum, and a progressive gross lymphocytic leucopenia.

(4) Resistance to artificial or natural cutaneous leishmaniasis is well-developed after the full course of infection. Superinfection results in synchronized healing of both primary and secondary lesions, varying with the host's state of resistance stimulated by the first infection, as stated in the "Reinoculation Law" of Moškovskij (1937).

(5) Resistance to visceral leishmaniasis is essentially absent once the splenic phase begins, much as in the Syrian hamster. Death is the usual result of untreated infection, resulting from an overwhelming lymphocyte hyperplasia. However, resistance is as strong after chemotherapeutic cure of visceral leishmaniasis as it is after the healing of a cutaneous leishmaniasis ulcer. Presumably protection against both depends upon skin sensitization and effective antibody concentration or lymphocyte activation.

(6) Cure or healing of either type of infection is indicated by a delayed sensitization tuberculin-type skin response (Montenegro or leishmanin test), which is, however, rare or absent in Indian kala azar.

Among the important questions still to be answered are the following:

(1) What is the specific role of plasmacytes and lymphocytes in the healing of cutaneous lesions?

(a) Do specific antibodies attack amastigotes only when they are released from destroyed

macrophages? Are these antibodies, which are released by sensitized lymphocytes, locally concentrated?

(b) Is there specific dissolution of infected macrophages by activated lymphocytes?

(c) Are the amastigotes attacked within the intact but presumably modified host macrophage or when infected macrophages are surrounded by sensitized lymphocytes?

(d) Is there a nonspecific histamine reaction and a walling-off of the lesion, thus isolating it during the course of an immune reaction?

(2) What is the stimulation and mechanism responsible for massive lymphocytogenesis? How is this related to the splenomegaly and hepatomegaly in visceral infection, in which local concentration of lymphocytes is combined with leucopenia in the general circulation?

(3) What is the source and stimulation for specific complement-fixing antibodies in visceral but not in cutaneous infection?

(4) What is the source and stimulation for the genesis of nonspecific serum gamma-globulin in visceral but not cutaneous infection?

(5) How do parasite and host share responsibility for parasite localization, which in cutaneous leishmaniasis causes restriction of infection to dermal macrophages and, in visceral leishmaniasis, involves a generalized reticuloendothelial invasion?

Visceral leishmaniasis follows a fairly well-defined progressive systemic course, essentially an all-or-none reaction. The range of cutaneous leishmaniasis, however, is from a minor non-ulcerating nodule, to an involvement of the entire dermis (as in leishmaniasis tegumentaria diffusa), or to the metastatic destruction of all nasal and buccal mucous linings and cartilage (as in *espundia*). Furthermore, except in *espundia* and individual intractable cases, cutaneous infection is self-limiting (or host-limited). How can we account for these differences?

(6) What is the relationship between degree of sensitization of dermal lymphocytes and plasma cells in the skin and control of cutaneous infection? How is this related to leishmaniasis recidivans? To leishmaniasis tegumentaria diffusa? To *espundia* of *L. brasiliensis*? To the kala azar dermal leishmanoid of *L. donovani* (a post-cure skin nodular reaction common in India)?

In few parasitic diseases are the problems, the challenges, and our ignorance of host-parasite interactions more obvious and more in need of clarification than in leishmaniasis.

#### NATURAL RESISTANCE OR SUSCEPTIBILITY

Man is one of the most susceptible hosts to all forms of leishmaniasis. The Syrian hamster (*Mesocricetus auratus*) is even more susceptible, and is apparently unable to develop any resistance during the course of an infection. Infections of *L. tropica* are strictly limited to the skin and spontaneously cured in man, but some strains will invade the viscera in hamsters and kill them. A similar invasive capacity exists for certain other rodents tested in the laboratory. On the other hand, white rats and rabbits will quickly eliminate an inoculum of *L. donovani* that would be fatal to man, hamsters, or the chinchilla (*Chinchilla panigera*). White mice will retain for life an *L. donovani* infection at a low level while the cotton rat (*Sigmodon hispidus*) can sustain a massive lifetime infection without apparent ill-effect. The guinea-pig, the clawed jird (*Meriones unguiculatus*), and white mice can tolerate and eventually destroy an infection. In these cases it is a matter of the speed and effectiveness of an innate host response. The capacity to resist infection or invasion of the viscera clearly varies among species and reflects more the influence of the host than that of the parasite. Human reaction shows a broad range of response, although the parasites do appear to differ in their inherent ability to invade the viscera.

Epidemic outbreaks of visceral leishmaniasis have taken thousands of lives and destroyed whole villages in Assam, Kenya, and the Sudan. The speed of the outbreak and the state of acquired immunity in the population were without doubt closely related to the epidemic patterns that developed.

Zoonotic cutaneous leishmaniasis has been reported from desert and semi-desert zones of Uzbekistan and Turkmenia in the USSR. In urban populations of Ashkabad in Turkmenia and in Kirovobad in Azerbaidzhan the anthroponotic type of cutaneous leishmaniasis was once common but is now extirpated. The distribution of infection in these people reflected the typical pattern of disease in an exposed population: young children of the indigenous population and persons of all ages among newcomers.

Mediterranean zoonotic kala azar caused by the *infanta* form of *L. donovani* is equally distinc-

tive. Infection is widespread among village dogs and in children is generally limited to those under 3, declining in frequency in older groups. A different age-distribution characterizes the anthroponotic form of kala azar in India, where adolescents and young adults comprise the most commonly infected group. In China, another zoonotic region, the picture is more like the Mediterranean one. These distinctive patterns indicate more than innate differences in the human populations. Each area also has its specific *Leishmania* vector. Each vector may interact in a specific way with the parasite's metabolism, development, and infection characteristics. Direct or indirect influences of the intermediate host on the promastigotes must be included in the host-parasite complex. Presence or absence of a vertebrate reservoir host and the cycling rate may also affect the virulence of the parasite in man, and determine the epidemiology of the resulting disease.

#### ACQUIRED RESISTANCE FROM NATURAL INFECTION

Resistance to cutaneous leishmaniasis following natural infection in man is generally considered to be 97–98% effective. Davydov (1963) reports a record 10% reinfection rate. This is an exceptional situation that developed in Turkmenia where 80–90% of the non-immunes were infected and as many as 50% of the sandfly vectors were positive. Immunity is a quantitative matter; therefore several lesions acquired during the susceptible period will confer a stronger immunity than a single lesion. Similarly, the more severe the lesion the faster the host responds, the quicker the lesion heals, and the stronger the immunity induced. Immune individuals who leave an endemic area and return some years later are still immune.

Immunity to visceral leishmaniasis is apparently absolute if the patient survives his first infection, that is, after chemical cure. But resistance prior to cure is seldom enough to control the initial infection. Immunity to mucocutaneous infection is much less certain. As with visceral leishmaniasis, there is probably strong resistance to a dermal challenge. But pockets of the original infection appear to escape and may metastasize years later to produce the destructive mucocutaneous lesions that are probably never completely cured even with chemotherapy.

The elimination of asymptomatic or avirulent infections in foci of endemic leishmaniasis has been

reported. This probably accounts for the failure of apparently susceptible individuals to become infected during mass outbreaks. Napier (1924) reported similar "spontaneous" cures among a significant proportion of Indian kala azar cases. Southgate & Oriedo (1967) and Southgate & Manson-Bahr (1967) found a number of Kenyans leishmanin-positive. A group of 13 volunteers among them successfully withstood the challenge of *L. donovani* from a human patient. Perhaps these Kenyans had had asymptomatic kala azar, or an infection restricted to a dermal lesion, as Cahill (1964) suggested in 4 American cases in the Sudan. On the other hand the local population is constantly exposed to natural infections with mammal or lizard leishmaniae. Such exposures over a long enough period might also stimulate an immunity to *L. donovani*, or cause the organism to be restricted to a dermal lesion. A significant proportion of those Sudanese with no history of kala azar who were skin-tested by Cahill showed a positive response. As in Kenya, the percentage of positives increased with age—an epidemiologically useful finding. Absence of cross-immunity between kala azar and other disease strains sharply differentiates it from the pattern of immunity against cutaneous leishmaniasis in the Soviet Union. A considerable degree of cross-reaction exists between *L. tropica major* and *L. tropica minor*, though a virulent full-course dermal infection is required to produce complete immunity. *L. t. major* infection can protect against both forms, but *L. t. minor* protects only against the relatively less virulent homologous strain.

#### ACQUIRED RESISTANCE FROM CONTROLLED INFECTION (VACCINATION)

The resistance to reinfection produced by the natural disease is most effective, as stated above, if the strain is a virulent one and the infection is allowed to run its full course. Interruption by treatment, surgery (Marcinovskij & Ščurenkova, 1924), or infection with a nonulcerating strain produces a corresponding diminution of protection. This is equally true of a vaccination infection. "Vaccination" against cutaneous leishmaniasis is a controlled but full infection with a freshly isolated virulent strain of living organisms, usually a zoonotic strain from gerbils or human patients.

Dr O. I. Kellina's careful laboratory studies and Dr N. F. Rodjakin's extensive trials with various

antigens demonstrate the need for a fully viable strain that will cause the patient to pass through the ulcerative and healing stages to ensure protection. Although Rodjakin reported at the Seminar that relatively virulent *L. tropica minor* can protect to some degree against *L. tropica major*, the more virulent zoonotic form is far more effective and has been used in the tens of thousands of inoculations performed in the Soviet Union. An avirulent inoculum will sensitize the skin to a leishmanin test, but it will not protect the individual against a more virulent strain.

The "Reinoculation Law" of Mořkovskij (1937) is particularly well illustrated in cutaneous leishmaniasis. If a challenge or second inoculation occurs during the course of a first infection, the second lesion quickly reaches the same stage of ulceration as that of the first ulcer, after which both sores heal synchronously. There is apparently no direct effect of one lesion on the other, but the first causes a reaction in the host that is responsible for a similar reaction against any subsequent infection. The rodent form of visceral leishmaniasis in Kenya also illustrates this principle. Reinoculation of volunteers with ground-squirrel *Leishmania* produces a nodule that heals concurrently with the preceding nodule, after which leishmaniae disappear simultaneously from both lesions. In Chiclero's ulcer of Mexico, however, immunity to a new infection develops very rapidly, so that a challenge inoculum may heal after the primary one has already healed.

#### CROSS-IMMUNITY

Some degree of cross-reaction develops between the strains of *L. tropica*, but, as noted above, *L. t. major* can break through the immunity barrier established by *L. t. minor*. Panamanian *L. tropica* protects against *L. mexicana*, but the latter does not provide protection against the former (Lainson & Shaw, 1966). In rhesus monkeys, *L. mexicana* protects against *L. brasiliensis*, but the reverse does not occur (Lainson & Bray, 1966). Sufficiently strong dermal involvement appears to stimulate cross-protection against related forms that produce a less acute cutaneous infection. Thus, if infection from *L. brasiliensis* could be reduced to a brief dermal one, fear of the terrible mucocutaneous after-effects that now strike about 80% of *L. brasiliensis* victims would vanish. *L. tropica* does not protect against *L. donovani*, though the visceral

form has been reported to prevent cutaneous infection. These reports require further investigation.

Immunization against visceral leishmaniasis has been attempted on a large scale only in Kenya by Dr P. E. C. Manson-Bahr, using Dr. R. B. Heisch's ground-squirrel strain of *Leishmania*. Whereas the cross-immunity experiment worked well among volunteers challenged with *L. donovani* cultures from a human case, vaccination with ground-squirrel *Leishmania* failed to prevent infection when transmitted in the normal fashion by sandflies. However, only about 20% of the "immunized" population were actually converted to a positive leishmanin response, so the immunization procedure followed in this large-scale clinical experiment was unsatisfactory. The possibility of protective immunization with an immunogenic strain that does not invade the viscera therefore is questionable.

The extent of cross-immunity between strains varies with infection conditions. Length of exposure to a primary infection appears to affect the result of a challenge by a second strain. This may help to explain some of the puzzling findings in cross-immunity tests between *L. tropica*, *L. brasiliensis*, and *L. mexicana*.

#### PASSIVE TRANSFER OF IMMUNITY

Immunity transfer from mother to child does not occur via milk-borne antibodies in cutaneous leishmaniasis (Gitel'zon, 1933; Rodjakin, 1957) and *L. enriettii* of guinea-pigs (Demina, 1964). Antibodies may well be transferred in this manner but, as in human visceral leishmaniasis, there is no evidence that such antibodies are functionally protective.

Adler & Nelken (1965) demonstrated passive transfer of delayed dermal hypersensitivity using human antiserum, but they failed to show successful passive transfer when they use whole blood or white cells taken from hypersensitized human donors. The whole blood or white cells were inoculated intradermally into a volunteer who then was challenged with leishmanin. Dr L. A. Stauber (personal communication) indicates that in his laboratory Dr Doisia has succeeded in demonstrating passive transfer of sensitivity in guinea-pigs inoculated intradermally with rabbit antiserum. Doisia found that the inoculation technique selected, the quantity of antiserum used, and the choice of host were crucial for the successful passive transfer of sensitivity. Dr K. J. Ott, another student of Dr Stauber, demonstrated reduction of a super-

infection in mice inoculated with guinea-pig anti-serum. Both findings are highly interesting and point to renewed interest in the serological aspects of visceral leishmaniasis.

#### SEROLOGICAL TECHNIQUES

The standard serological tests that are available emphasize the failure of leishmaniasis to follow a pattern suitable for conventional microbial serology. Antibodies cannot be detected in most cases of cutaneous leishmaniasis, as in many other intracellular and tissue parasitic infections, such as leprosy, tuberculosis, and infections with various metazoan parasites. In visceral leishmaniasis, positive serological findings are not correlated with the state of immunity.

Agglutination and complement-fixation tests are relatively nonspecific in leishmaniasis, though the CFT is useful as a diagnostic technique, particularly in canine visceral leishmaniasis, where cross-reaction with Chagas' disease is not significant.

The fluorescent antibody test is group-specific and loses its reactivity after absorption of common antibodies.

Demina (1962) showed that promastigotes of *L. tropica* or *L. enriettii* can be kept for long periods in immune serum and do not lose their pathogenicity to mice or guinea-pigs. The organisms do, however, develop characteristic growth patterns in antiserum, which appear to be a sensitive measure of relationship. This phenomenon, first noted by Noguchi (1924), was further developed as a diagnostic test by Adler (1963, 1964). The antiserum-culture test is the only procedure at present available for determining species relationship among the leishmaniae. It demands great care in preparation of antisera, however, and requires the use of a number of rabbits to prevent the serum from showing a nontypical reaction. It is necessary to observe growth patterns over long periods to detect the precise differences among promastigote colonies grown in various dilutions and kinds of antiserum. Lack of standardization of procedure and interpretation of results is still a major limitation to its wider adoption. Prior absorption of rabbit antisera against cross-reacting species of *Leishmania* enhances the sensitivity of the test. Adler was able to differentiate Mediterranean from Indian *L. donovani*; *L. tropica major* from *L. t. minor*; and *L. donovani* from *L. brasiliensis*, *L. mexicana*, and *L. tropica*. Saf'janova (1966, 1967) has worked out a complex

set of measurements for titrating results with this test. She made a valuable comparison of various lizard strains with rodent and human strains of *L. tropica major*; Dr M. de V. Coelho applied the technique to the New World forms, the results indicating that "*L. pifanoi*" is a form of *L. brasiliensis*. Studies are also under way at the Wellcome Parasitology Unit in Belem, Brazil, where Dr R. Lainson is investigating the difficult New World *L. tropica* complex.

Bray & Rahim (1969) have developed a promising haemagglutination test in Iraq, using sheep cells sensitized with polysaccharide extracts of the tested parasites.

Professor O. Theodor has suggested that sensitive immunoelectrophoresis and diselectrophoresis procedures might differentiate species of *Leishmania*, as they have malaria parasites.

Schneider & Hertig (1966) used a micro agar-gel immunodiffusion test at the Gorgas Laboratory in Panama. They studied 5 human strains and 11 sandfly strains—8 from local sandflies and 3 from South America. Two distinct groups were characterized, but these were not related geographically or with specific sandfly hosts. Common antigens again masked the results.

Rodjakin & Khanmamedov (1967) developed a promastigote immobilization test, which they feel is a very promising research technique.

There is still a need for a simple laboratory test to identify species against type colonies using standardized procedures, preferably in a leishmaniasis reference laboratory. The tests mentioned here remain primarily research techniques, difficult to interpret, and cumbersome or demanding to process on a scale large enough for routine use.

#### NONSPECIFIC TESTS

Visceral leishmaniasis stimulates hypergammaglobulinaemia to a degree that globulin change in serum can be detected by several simple, inexpensive, nonspecific tests. These have been used successfully in India for some years, where they serve as a rapid screening method for preliminary diagnosis. The tests include Bramachari's reaction, Napier's test, Chopra's test, and Sia's reaction. Hypergammaglobulinaemia, though characteristic of visceral leishmaniasis, is not confined to it and is not correlated with antibody level. Demina believes that this condition may also indicate auto-antibodies, formed in response to the presence

of damaged host cells that are thereafter recognized by the body as foreign substances. Šujkina has made an extensive paper electrophoretic and refractometric study of human and guinea-pig sera after subjecting the hosts to repeated doses of leptomonads. No abnormal changes could be detected in protein levels or in differentiated white cell counts. The fluorescent antibody test with these sera was negative or slightly positive. According to Vasina, Demina & Glazunova (1965) guinea-pigs infected with *L. enriettii* demonstrated a sharp increase in plasma cells in the spleen and in lymph nodes at various stages of the infection and after reinoculation. This evidence, in addition to that from delayed hypersensitivity studies, suggests that cell-bound antibodies are involved in the host's response, even in visceral infections. This aspect is in particular need of clarification.

#### SKIN TESTS (MONTENEGRO REACTION)

This test is a standard delayed sensitivity intradermal test of the allergic or tuberculin type. Killed promastigotes of any species, even *Strigomonas* or *Trypanosoma*, will serve as antigen, indicating a common-antigen basis of the response. Tuberculosis of the lymph nodes and leprosy also react positively, but the test is nevertheless useful as a diagnostic aid and a measure of previous exposure of residents of endemic areas. According to Moškovič, it was found that in people who did not contract the disease the skin test was positive only after a sojourn of not less than 5 years in an endemic area. The reaction is probably lifelong; its duration has been reported as at least 35 years (Adler, 1961), 35 years (Kellina), and 21 years (Rodjakin). Extensive skin testing, aided by statistical age-prevalence studies, might help to determine the initial onset of disease in a community or demonstrate past epidemics. However, high infection densities may mask any such interpretation. Furthermore, the response of persons living in an endemic area to continual exposure to animal leishmaniae may add to the complications. Both sources of confusion appear to have taken place in Kenya (Manson-Bahr, 1961) and in the Sudan (Hoogstraal & Heyneman, 1969).

Use of the Montenegro reaction is particularly important in the diagnosis of occult *L. brasiliensis* infection or in cases of mucocutaneous involvement where it is frequently difficult or impossible to demonstrate the organisms.

A positive leishmanin response may appear very soon after exposure to *L. mexicana* or *L. brasiliensis*, 10–20 days after a successful inoculation with *L. t. major*, 6 months after inoculation with the milder *L. t. minor*, and about 2 years after cure of visceral infections in Africa. In India, conversion to a positive skin test after kalaazar does occur, according to Professor P. C. Sen Gupta, though the conversion is slow to develop and variable. The dermatotropic characteristic of African visceral leishmaniasis is suggested by random surveys. A positive skin test response has been found in a large proportion of the Africans in endemic regions of Kenya and the Sudan. Inability to sustain a skin hypersensitization, the normal prelude to immunity against leishmaniasis, is indicated in individuals who do not show a skin test response after cutaneous infection (as in "diffusa" patients).

#### DEMONSTRATION OF NATURAL VARIATION IN VIRULENCE AND ITS ROLE IN IMMUNIZATION

Kellina has studied many rodent and human *L. tropica major* strains in order to compare their virulence and antigenic effectiveness as vaccines. By careful control of infection procedures and standardization of techniques, she was able to demonstrate a remarkably broad spectrum of virulence among many strains within a single *Leishmania* subspecies. The innate virulence of each strain was tested in white mice, golden hamsters, gerbils, and man. The susceptibility of the hosts to infection and the sensitivity of the hosts were determined. Animals were tested for number of parasites, length of incubation, speed of development of infection, maximum intensity, and frequency of invasion of the viscera. Human volunteers were checked for the proportion of persons infected, the duration of incubation, the time to ulceration, the duration of ulceration, the size of the scar, and the effectiveness of protection. Thirty human strains were studied (22 from Turkmenia by Kellina & Belova and 8 from Uzbekistan by Ni). All the Uzbek human strains were highly virulent, as were two strains from north-east Iran studied by Ansari (1947) and Ansari & Faghieh (1953). All human strains showed some degree of infectivity in mice.

In all, 39 rodent strains were studied—26 from Turkmenia and Uzbekistan by Kellina and 13 from Uzbekistan by Ni (1967). They demonstrated a broader range of virulence than did the human isolates. Some of the least virulent ones, in fact,

failed to infect mice at all, although this infectivity is a basic criterion for definition of the zoonotic *L. t. major* subspecies. All rodent strains tested were placed in one of three groups according to virulence: high, low, and intermediate. Subsequent experiments performed with many fresh strains isolated in the USSR from man and rodents fully confirmed these results. The criteria employed by Kellina for allocation of the rodent strains to the 3 groups are listed below:

*Group I:* high virulence (9 strains that infected all 3 laboratory hosts)

- (1) Lesions are produced in 80–100% of mice;
- (2) lesions appear after a short incubation;
- (3) lesions develop progressively from papule to a non-healing ulcer;
- (4) invasion of the viscera is common and secondary foci often appear in limb joints;
- (5) infiltrates appear on the ears of gerbils, with loss of hair and tissue;
- (6) hamster infection is fulminating, with rapidly ulcerating ear lesions and progressive invasion of the viscera, ending fatally.

*Group II:* low virulence (28 strains)

- (1) Only a proportion of mice become infected;
- (2) lesions are mild and short-lived;
- (3) hamsters are more susceptible than mice; all develop chronic nonulcerating infiltrates on the ears.

*Group III:* intermediate virulence (2 strains)

- (1) Only a proportion of mice become infected;
- (2) infected mice develop characteristic progressive lesions;
- (3) long incubation is required and growth of papules is slow, though they eventually ulcerate.

(Group III response was similar to that seen in a strain recovered from *S. arpaklensis* by Šišljaeva-Matova et al., 1966.)

In the human inoculations, Kellina considered the following to be due chiefly to the host's response:

- (a) size of lesion;
- (b) degree of inflammation;
- (c) depth of tissue disintegration;
- (d) abundance of discharge; and
- (e) number of secondary papules.

The structure of the patient's skin appeared to be of particular importance in determining the intensity of reaction.

The factors that Kellina considered to be due chiefly to the parasite strain were:

- (a) proportion of persons infected;
- (b) incubation period;
- (c) speed of ulceration; and
- (d) number of promastigotes needed for infection.

In certain individuals, strains that generally produced an acute infective process nevertheless caused a surprisingly prolonged development of the lesion, usually a characteristic of low virulence.

Kellina also distinguished 3 groups of parasite strains based on the response of the human host:

*Group I:* high virulence

- (1) Nearly 100% of persons exposed are infected;
- (2) papule forms in 1–3 weeks;
- (3) papule softens and ulcerates in 3–6 weeks;
- (4) ulcer heals 3–4 months later.

*Group II:* low virulence (1 strain)

- (1) Not infective to man.

*Group III:* intermediate virulence

- (1) Infection occurs only in certain hosts;
- (2) infective process is weak and brief;
- (3) no ulceration develops;
- (4) resorption occurs in 4–6 weeks with no subsequent scarring.

#### ALTERATION OF VIRULENCE

Even the highly virulent cultures tested by Kellina lost some virulence after prolonged cultivation. Some strains even lost virulence after maintenance in vertebrate hosts without a sandfly phase. This loss ranged from none, as in a strain that was still virulent to man after cultivation for 31 years (Adler, 1961), to a very rapid loss that occurred after 3–5 transfers (Rodjakin, 1961).

Kellina reported that she slowed the loss by using solid NNN culture medium without antibiotics, transferred only once a month. Most strains remained unchanged for 2–3 years. One virulent strain withstood 30 passages during 2½ years, but after having been sent to London it lost virulence in 10 weeks on a liquid medium



that was frequently changed (Neal, 1964). After subculture for a long time, even on solid media, the virulence of most strains fell. The loss of virulence was seen first in mice and later in man. Abortive or mild chronic lesions could still develop in man after inoculation with a strain that had become noninfective to mice. Essentially it had transferred from a zoonotic to an anthroponotic strain.

Virulence was later recovered by selection of active lesions from test mice. It could be sustained by this means for long periods (7–8 years for the virulent strain P). By artificial selection from severe lesions, Kellina raised two strains from Group III to Group I.

The natural heterogeneity of *L. t. major* seems well-established. Doubtless this fact has played an important role in natural selection as well. The results of tests with mice are similar to those of tests in man. The virulence of strains can be modified by continual selection. Since mice are more resistant to infection than man, Kellina could detect the loss of virulence in mice before it was possible to see any difference in human infections. This difference proved to be useful in the repeated standardizations required for the development and use of a leishmaniasis vaccine.

#### PREPARATION OF VACCINES

Kellina's work made it clear that an attenuated strain of low virulence was not suitable for human protection. Cultures of sustained high virulence must be used in order to produce a quick-ulcerating, short-lived infection, able to immunize against any challenge from zoonotic *L. t. major*. A milder form will induce sensitization, but will not ensure protection from ulcer formation by a stronger pathogen. Even after proper protection, reinoculation with a highly virulent challenge by natural exposure or by experimental inoculation will still produce a mild biphasic response: (1) a delayed allergic reaction develops first; and then (2) papule formation occurs in the second week, with a very small non-expanding ulceration that may last for several weeks or months before complete resorption or formation of a small scar. It is interesting to note that Kellina demonstrated live *Leishmania* from these immune sites up to the 22nd day.

The inoculation procedure, as developed in the USSR, produces a natural but controlled infection

in a covered site without disfigurement, crippling, or loss of working time. Rodjakin has reported that no generalized spread of the inoculum has ever occurred among many thousands of successful vaccinations. Growth confined to the skin is an intrinsic trait of *L. tropica*, and there is no danger of visceral involvement according to the Soviet workers.

The cultures used by Rodjakin for the production of vaccines are grown in 150-ml bottles on NNN medium with 1000 units of penicillin and 1000  $\mu$ g of streptomycin. The surface condensate is transferred to 3–5-ml ampoules after 5–6 days. Stored vaccination materials can be used for 8–10 days.

In performing the vaccination, considerable care must be exercised to ensure that the skin area selected (right hip or scapula) is properly disinfected and that the instruments are aseptic, in order to prevent sepsis and ensure proper growth of the promastigotes that are inoculated. Intracutaneous inoculation with a tuberculin syringe must be used and the volume of the inoculum should be 0.1–0.2 ml.

The stages of development of the vaccination ulcer are as follows:

(1) The incubation period is from a few days to 3 months (average 34 days);

(2) A hyperaemic spot appears, solidifies, and increases in size in 5 days to a nodule. Enlargement of the papule follows. It becomes flattened, thin at the surface, and then ulcerates (average period 37 days);

(3) Asymmetric ulceration follows decomposition of the nodule. The infiltration zone is small. The diameter of the ulcer may reach 1–1.5 cm, but seldom exceeds 0.5 cm (average duration: 82 days). The average total reaction time is 118 days.

The more acute the clinical manifestation is, the briefer the ulceration. The vaccination ulcer, in the experience of the workers in the USSR, never reaches the size of a natural lesion, nor does it induce tissue destruction and other complications that often ensue after a naturally acquired infection.

Ultimate control of the disease requires control of the zoonosis. Immunization is employed only to reduce the risk of injury and loss of time for those who are exposed in endemic areas. Eradication of the focus would require a broad-scale attack on reservoir hosts, vectors, and human infections. Such multiphase measures have already eliminated urban leishmaniasis from the Soviet Union and have reduced the zoonotic forms to a few isolated foci.

## SPECIES AND SEROLOGY

Species identification among the leishmaniae is of considerable interest and causes immense frustration. Morphological criteria are unreliable and essentially useless. Epidemiological factors, host and vector species, and the range and cycling rate of different strains are all without doubt very important in the process of species formation. But these criteria are difficult to quantify and compare, especially with new strain isolates whose life cycles are unknown. *In vitro* serological tests remain the most reliable index, but these are far from satisfactory. Yet, new forms of *Leishmania* continue to appear, to be described, and to require clarification. Are they genetically distinct? Do their disease characteristics reflect that uniqueness? Are these characteristics constant?

The answer is of more than biological interest. It will help to govern our handling of the disease or to prevent the risk of exposure of nonimmune populations. The possibility of invasion of the viscera or mucocutaneous destruction greatly modifies the control or clinical measures to be selected. A change of parasite strain often results in a change of pathogenesis. The identification of such a change is a function of our knowledge of the species involved. The validity of *L. tropica major* and *L. t. tropica (= minor)* as subspecies is a good example and is the subject of much discussion. Uncertainty over this division has been reflected in an uncertainty over the proper handling of individual infections and of exposed human populations in endemic areas of cutaneous leishmaniasis outside the Soviet Union. Even within the USSR, Kellina's discovery of heterogeneity in the zoonotic complex of rodent strains of *L. t. major* emphasizes the great speciation potential in this complex, ranging from strains that infect mice at a very low level if at all to those that rapidly kill all mice tested. The loss of virulence in culture and its subsequent recovery by passage through laboratory rodents emphasize the fact that these organisms retain a considerable degree of evolutionary plasticity.

The considerable degree of cross-immunity that exists between anthrophilic and zoonotic strains illustrates their basic similarity. Clinical variations show a broad and confusing overlap that prevents clear-cut separation of strains on clinical grounds. Yet Adler (1963, 1964) could distinguish *L. t. major* from *L. t. minor* by his culture-antiserum method. Admittedly the difference between the

strains was slight and the method required the comparison of a number of *L. t. major* strains rather than with only one and also required intimate familiarity with the technique. Are the various strains we study in the laboratory simply different expressions of a genetic continuum, or do they constitute genetically distinct or nascent types? We assume that *L. t. major* changed to a mild pathogen, *L. t. minor*, when after sufficient selection and isolation, it became part of an urban focus with a new vector and the loss of its rodent reservoir. On the other hand, might not *L. t. minor* increase its virulence again if it were passed long enough through dogs or urban rodents? If the patterns of pathogenicity now recognized in man are a reflection of genetically distinct parasite complexes, we would be justified in a fairly rigid taxonomic and clinical codification. But separation between such units tends to break down, particularly at the subspecific level. Intermediate forms are found, criteria lose their clear borders, and host effects confuse the distinctions. The mid-grouping of each cluster of geographical strains is usually clear, nevertheless, and the division is epidemiologically useful, as in the New World agents of cutaneous leishmaniasis. However, the question of biological (i.e., genetic) distinction among the many forms of *Leishmania* found in man is no closer to solution today than when it was argued 50 years ago.

Similar questions will continue to arise as still more variants and intermediates are described. *L. mexicana* can be separated on serological grounds. The relatively nonvirulent *L. peruana* and *L. guyanensis* can be separated on geographical and epidemiological grounds, and should be retained as a matter of clinical and biological usefulness pending serological review. The Panamanian strains discovered by Hertig and co-workers (which may include non-mammalian flagellates), and those isolated by Strangeways-Dixon & Lainson in Belize, British Honduras, must also be evaluated.

*L. chagasi*, in contrast to the species mentioned above, stands in doubt, even though no type of visceral leishmaniasis other than that caused by this organism has been found in South America. It appears to be an introduced form with an epidemiology closely tied to the domestic dog, whose infections may have spilled over into the adjacent wild fox population (*Lycalopex vetulus*). This view suggests that the domestic dog is the source and not the recipient of the disease from wild

reservoirs. According to Garnham the matter is not resolved and an intensive search for a wild animal reservoir is required before we can dismiss the view that an endemic New World form of visceral leishmaniasis exists, one that antedates human occupation of Brazil. Infection is transmitted chiefly if not entirely by *Lutzomyia longipalpis*. Serological separation of *L. chagasi* from *L. donovani* is not possible by the Adler technique, which suggests that *L. chagasi* is a recent genetic variant of *L. donovani*, or one that is strikingly similar to it if *L. chagasi* is a truly endemic species. Based on present evidence, however, specific status is not yet warranted.

Adler et al. (1966), using Adler's culture technique, also confirmed the serological (hence genetic) cross-specificity of the various strains of *L. donovani* found by Hoogstraal and co-workers in the Sudan. Strains were isolated from man, from the roof rat (*Rattus rattus*), from the Nile rat (*Arvicanthus nyloticus luctuosus*), from the spiny rat (*Acomys albigena*), from the serval (*Felis serval philippsi*), and from the genet (*Genetta genetta senegalensis*). This broad host spectrum suggests a relatively non-host-specific strain. It also leaves unanswered the question of its origin and status as an anthroponosis or a zoonthronosis. It could be a fairly recent immigrant, presumably from India via Arabia, Ethiopia, and Kenya, which then infected a large number of vertebrates, including man (hence it would be a zoonthronosis). Or it might be a truly endemic zoonosis, found in many wild hosts and accidentally encountered by man (hence an anthroponosis). The question may not be answerable. The view favouring immigration is suggested by the recent history of kala azar epidemics in Kenya and the Sudan and the presence of the disease in Ethiopia, with epidemiological characteristics suggestive of Indian kala azar. Yet the large number of host species incriminated in the Sudan, involving both rodents and rodent-eating carnivores, is equally suggestive of a complex, widespread zoonosis. Evidence of a nonhuman cycle of infection was found in Malakal, in the central Sudan (Hoogstraal & Heyneman, 1969).

#### PREMUNITION VERSUS IMMUNITY

The question of a premunition, or non-sterile protection (the occult presence of a primary infection), as opposed to a true residual (sterile) immunity is not yet solved. Evidence of long persistence of the

agent in man is quite convincing. "Live" scars have been described by Koževnikov (1941) and Koževnikov et al. (1947), Šubarova (1957), and Latyšev, who found living organisms in "healed" cutaneous lesions 6 months, 4 years, and 13 years, respectively, after cure. Fluctuating erythema, peeling, oedema, and nodule formation around healed ulcers indicate survival of infection following the supposed cure of a cutaneous lesion. Parasites have been cultured from the tissues of dogs (Vavilova, 1960) and gerbils (Krjukova, 1941) presumed to be cured of infection, and similar cultures have been obtained from laboratory rodents presumed to be cured of visceral infections (Stauber, personal communication). The clinical history of *espundia* demonstrates long parasite survival and eventual metastasis from foci presumably hidden in the skin, possibly hidden in nasal mucosa as well. Buccal and nasal tissues may be attacked by *L. brasiliensis* 7 or more years after the original lesion has healed. Post-kala-azar dermal leishmanoid often develops several years after the cure of visceral leishmaniasis, as has been reported recently by Dr P. C. Sen Gupta. This creates an enigma. Visceral "cure" is followed by dermal resistance to a new infection. But, after a varying period, skin resistance is lost, and a resurgence of the old infection develops as a papular dermal outbreak, while the original visceral organs remain uninfected. These nodules, which are varied in form and frequently highly resistant to treatment, contain numerous parasites. Dermal leishmanoid sometimes spreads throughout the dermis, much as in "diffusa" cases of cutaneous leishmaniasis. Similarly, the dermal infection known as the "recidivans" type is a recrudescence of the active spread of infection at the margins of old "healed" leishmanial scars. Frequently these infections resist all attempts at chemotherapy, smouldering on for decades.

Reports of asymptomatic visceral infection (Armstrong, 1945; Prata, 1957; Sen Gupta, 1962), as well as positive leishmanin tests in East African and Sudanese endemic centres of visceral leishmaniasis, add to our uncertainty over the existence of a true sterile immunity. Premunition may therefore exist in many individuals, and constitute a continuous source of antigen stimulation in the dermis. The presence of functional immunity in many persons free of parasites is nevertheless equally clear.

Patients who have recovered from kala azar, or who have been cured by chemotherapy, appear

to possess a solid immunity against reinfection, even in hyperendemic areas where re-exposure is frequent. But this protection is not related to the high CFT titres and hypergammaglobulinaemia that develops during the course of infection. These serological manifestations seem unable to serve any protective role against the course of visceral disease in man.

In animal experiments with *L. donovani*, chiefly by Stauber and his students (Stauber, 1955, 1958), an immunological element in resistance is inferred rather than proved directly. Rabbits, white rats, guinea-pigs, gerbils, and white mice show a wide range of innate resistance. Some host species tolerate infection only at low levels, later arresting the infection and rejecting it; others cannot even be infected. At the other extreme, hamsters and chinchillas die of the infection, and the cotton-rat survives with a level of parasitism that is lethal to the hamster. Mice, guinea-pigs, and gerbils lose a challenging infection more quickly than they do the initial one. This rapid loss can be interpreted either as a degree of acquired resistance or as a premunition effect, as the initial infection was still present at the time of challenge. Comparative studies on reinfection after chemical cure remain to be carried out. Interference with the host's protein metabolism and reduction of antibody synthesis by elimination of pyridoxine in the diet drastically reduce both innate and acquired resistance to *L. donovani* in the white mouse (Actor, 1960).

#### FUNCTIONAL IMMUNITY AND HYPOTHESES OF THE IMMUNE PROCESS IN LEISHMANIASIS

Once human infection with kala azar passes the dermal barrier, the concentration of cellular antibody in the dermis is apparently useless in retarding infection. However, the host is protected against subsequent sandfly exposure following cure, as was noted in the preceding section. The hypothesis, therefore, seems warranted that immunity to visceral leishmaniasis resides largely in the skin, where cell-bound antibodies, plasma cells, and sensitized lymphocytes are concentrated. Experimental proof of this view is lacking, but it might be tested by comparing intradermal with intraperitoneal or intracardiac infection in a chemically cured animal. The animal of choice would be one that demonstrates some degree of innate resistance, such as the guinea-pig or *Meriones*, as shown by Stauber (1958). Positive results would not necessarily prove the case

for man, but the implication would nevertheless be strong.

The relative effectiveness of an immunizing infection in stimulating rejection of a subsequent challenge exposure appears related to the speed and degree of skin reaction. The host's capacity to respond, at least in cutaneous leishmaniasis, requires specific, intense, and prolonged stimulation. Kellina has emphasized that only a highly virulent infection allowed to develop through its full course would provide protection against an equally virulent challenge. Attenuated or less naturally virulent forms of *L. tropica* that fail to produce an ulcer do not stimulate full protection, despite earlier reports of success with killed leptomonads by Pessôa (1941) and others. Rodjakin's extensive practical experience in developing and using the *L. t. major* vaccine corroborates Kellina's experimental findings.

Yet Manson-Bahr (1961, 1963) demonstrated in a small number of soldier volunteers that protection is possible against the East African strain of *L. donovani* that invades the viscera using an intradermal inoculation of the relatively nonvirulent ground-squirrel strain that does not invade the viscera.

Subsequent field trials with 1500 persons in a Tana River endemic focus unfortunately failed to protect them against natural kala azar infection, owing to failure of the inoculation procedure employed. This may have been because the ground-squirrel strain used was impaired by long *in vitro* cultivation. Southgate has reported that he had repeated the immunization with the original ground-squirrel strain in Kenyan volunteers and that it did protect them against *L. donovani*.

The role of the skin as the major defence organ against leishmaniasis suggests some comparative observations between Indian kala azar and other forms of visceral leishmaniasis:

(1) In Indian kala azar, evidence of a primary lesion is rare or absent, whereas it is common in Sudanese and East African foci and is probably present in other areas as well (e.g. in Uzbekistan).

(2) Demonstration of parasites by direct microscopic blood examination is common only in Indian kala zaar. In Kenya and the Sudan, parasitaemia during early stages of infection is equally common but less intense, and is detectable only in cultures of blood. On the other hand, the isolation of parasites from skin snips or nasal mucosa is common in African and rare in Indian cases.

(3) Post-kala-azar dermal leishmaniasis is common following Indian kala azar (5–10%), but rare or absent in other areas with the possible exception of China. Sen Gupta has said that the numerous cases reported from the Sudan and the few from Kenya are concurrent cutaneous manifestations, rather than true post-kala-azar dermal leishmaniasis as he defines it.

(4) Response to the Montenegro test is immediate and strong following the cure of African or Mediterranean kala azar. Sen Gupta has stated that it does occur after treatment for Indian kala azar, but that it is weak and slow.

(5) Indian kala azar responds readily to treatment with antimony (III) sodium dimercaptosuccinate (stibocaptate), which is ineffective against African forms and relatively so against the forms found in Mediterranean, Soviet, and Chinese foci.

(6) Spontaneous cure is reported to be common in Indian kala azar, averaging 10% and ranging up to 25%, according to Napier (1924). It is thought to be significantly less, perhaps 5–10%, in other areas.

Could these conflicting characteristics of what is considered to be the same infective agent be caused by a weaker dermal sensitization and consequently a lowered functional immune response in Indian kala azar? In the latter infection, the lack of a primary dermal ulcer and the abundance of parasites in peripheral blood is in contrast to the characteristically strong initial dermal response and lower parasitaemia in kala azar from other areas such as Africa. The greater number of parasites in the blood in Indian kala azar presumably renders it more amenable to blood-borne chemotherapy; the frequent appearance of post kala azar dermal leishmanoid and a less intense leishmanin response suggest a failure of the skin response.

Manson-Bahr (1961) noted that though the leishmanin reaction develops only after cure of East African kala azar, the reaction is positive much earlier if the infection does not invade the viscera. Similarly, non-rodent strains that do not invade the viscera induce a rapid leishmanin response, as does infection with *L. t. major* or *L. mexicana*. *L. t. minor*, which causes a less intense skin infection, requires about 6 months to induce a positive leishmanin response. These observations reinforce the view that it is in the skin that the most effective protection of man probably occurs against any form of leishmaniasis. Once infected macrophages escape from the skin, there is little chance for

the body to isolate and destroy them in spite of the massive proliferation of lymphocytes. Obstruction and enlargement of the liver and spleen result from this proliferation, inducing conditions that lead to profound hepatosplenomegaly and the death of the patient. Extreme hypergammaglobulinaemia appears to be a systemic recognition that something is wrong, but the exact nature of the disturbance is hidden from the immunologically competent cells—hidden, of course inside the phagocytic histiocytes. This undirected and ineffectual emergency defence response may be further increased if antigens from destroyed macrophages stimulate an auto-immune response. Perhaps this is what occurs late in the infection sequence in *espundia*, the final self-destructive stage of mucocutaneous leishmaniasis. Parasites are seldom seen at this stage, but nasal and buccal tissues are destroyed nevertheless. The body's defensive cells appear to have lost the vital characteristic of self-recognition. Destruction of the body's own tissues may occur rapidly once the process begins, as in other auto-immune wasting diseases. Damage to infected macrophages may have caused these cells to become antigenic, stimulating cell-bound antibodies or activated lymphocytes to attack other macrophages and ultimately the nasal mucosa and cartilage as well. We have no idea, however, why nasal and buccal mucosa should become the primary target, nor do we understand the role of the parasites in stimulating or directing the process. The frightfully disfiguring results are evidence enough of its importance.

Perhaps the key questions remain unasked for lack of a suitable experimental design. Why do infected macrophages remain in a cutaneous sore in one case, and a histiocytoma in another, and why are they not at all confined in a third? What determines blood- or lymph-borne metastasis to the nasal mucosa in one disease, or filtration within the liver or spleen in the other? Is this migration undertaken by freed parasitic cells or by infected histiocytes? These are all restatements of the same basic question: to what degree is the clinical form of leishmaniasis an expression of the parasite's genetic constitution (virulence) or of the host's innate capacity to respond (immunity)? The unsolved problems and unasked questions about this complex of diseases demand our continued interest and attention. The discussion of immunological aspects in these hypothetical terms is imposed by our ignorance and is an expression of that challenge.

## RÉSUMÉ

## IMMUNOLOGIE DE LA LEISHMANIOSE

La leishmaniose humaine résulte d'une interaction entre l'hôte et le parasite: le pouvoir envahissant intrinsèque du parasite et la virulence telle qu'elle est modifiée par la réponse individuelle et l'espèce de l'hôte. Le présent article passe en revue les aspects conceptuels et cliniques de l'immunologie de la leishmaniose humaine.

Nos connaissances sur les aspects immunologiques de cette affection restent fragmentaires et surtout pragmatiques, comme dans le cas de la vaccination anti-*L. tropica*. La variabilité de la réponse d'une même souche parasitaire chez différents hôtes est révélée par l'exposition de divers hôtes de laboratoire à des infections expérimentales normalisées. Chez l'homme, des réponses individuelles extrêmement variables masquent et compliquent la réponse de l'espèce. Celle-ci se produit, toutefois, ainsi que le montre la distribution régionale des formes cliniques de la maladie et les poussées épidémiques de kala-azar en Inde et en Afrique. On ne sait pratiquement rien de la mesure dans laquelle vecteur et réservoir contribuent à la maladie humaine.

La résistance acquise naturellement et les résultats de la vaccination mettent en évidence que seul le déroulement complet de l'infection par une souche virulente de *L. tropica* confère la protection contre une réinfection par une forme d'une virulence comparable. D'après des chercheurs soviétiques, seules des souches zoonotiques vivantes, récemment isolées, sont assez fortes pour induire une protection totale. L'évolution dermique de l'infection immunisante doit être rapide, aiguë et ininterrompue par un traitement. Une fois que l'immunité contre la leishmaniose cutanée a commencé d'apparaître, les inoculations d'épreuve en d'autres points de la peau provoquent des ulcérations qui guérissent en même temps que la lésion primaire (« loi de réinoculation » de Moskovskij). Dans la leishmaniose viscérale, l'immunité n'apparaît qu'après une guérison spontanée ou résultat d'un traitement médical.

Au cours d'études sur la protection croisée, on n'a pas réussi à juguler l'infection due aux espèces les plus pathogènes telles que *L. brasiliensis* et *L. donovani*. Il se pourrait qu'il y ait une exception importante, à savoir la souche qui affecte les rongeurs, ne provoque pas la leishmaniose viscérale et existe au Kenya, souche que l'on croit étroitement apparentée à *L. donovani* et qui a protégé des volontaires contre une inoculation d'épreuve par une culture de laboratoire d'une souche humaine virulente de *L. donovani*. Des études sur le transfert passif de l'immunité ont montré combien il est difficile de mesurer, ou simplement d'évaluer la résistance à l'infection cutanée. Bien qu'ils ne soient pas spécifiques, les procédés fondés sur l'étude du sérum sont utilisables pour le dépistage préliminaire. L'élévation de la gammaglobulinémie et des titres d'anticorps non spécifiques fixant le

complément sont des signes de kala-azar, mais ils paraissent indépendants de la gravité de la maladie ou de l'état d'immunité fonctionnelle. Le test cutané de Monténégro est utile dans le diagnostic de l'infection cutanée et il est important du point de vue épidémiologique dans certaines régions où le kala-azar est endémique, mais ses possibilités d'utilisation dans la pratique sont très limitées. Des chercheurs soviétiques ont étudié les différences de virulence entre les souches de *L. tropica major*, la modification de la virulence par culture *in vitro*, et l'application des résultats de ces études à la préparation de vaccins. La base sérologique de la séparation des espèces est encore loin d'être satisfaisante. La méthode d'Adler de culture par antisérum est peut-être la plus prometteuse, mais les procédés de culture, les souches, les antisérums et les critères d'interprétation des résultats ne sont pas complètement normalisés, d'où la difficulté de répéter les expériences dans d'autres laboratoires.

Malgré de nombreuses d'années d'efforts, personne n'a encore mis au point une définition satisfaisante de l'espèce *Leishmania*. Ce sont les critères épidémiologiques qui sont les plus utiles et les plus acceptables, car ils comprennent la variété maximale d'indices des relations parasitaires: facteurs géographiques, vecteur, réservoir et caractéristiques cliniques chez l'homme et chez les animaux d'expérience. Le complexe d'espèces d'Amérique du Sud et d'Amérique centrale a tout particulièrement besoin d'être élucidé. Les observations effectuées donnent à penser qu'il existe un certain nombre de formes de *Leishmania* dans le Nouveau Monde, principalement des zoonoses en associations hôte-vecteur-parasite dans des régions forestières tropicales écologiquement isolées; il s'agit, semble-t-il, d'espèces en existence ou en formation.

Il importe de prévenir la leishmaniose humaine. Les crypto-infections paraissent répandues et pourraient être partiellement responsables de la résistance contre la réinfection, que l'on constate parmi certaines populations des régions d'endémicité. La survie à long terme des parasites s'observe dans la leishmaniose récidivante (inflammation marginale d'une ancienne lésion), dans la leishmaniose tégumentaire diffuse (extension anarchique de lésions dans le derme) et dans la leishmaniose américaine ou « espundia » (destruction étendue de la muqueuse bucco-nasale et des tissus adjacents). Cette dernière peut impliquer une auto-immunité et les autres peuvent être dues à une allergie ou à quelque autre échec de l'immunité de l'hôte. Il est également probable que dans les régions d'endémie de nombreux individus hébergent, au niveau du derme, des colonies de parasites non pathogènes qui échappent à l'observation. Ces colonies peuvent prévenir la réinfection, mais être capables d'une recrudescence d'activité pathogène ultérieure.

Le rôle de la peau est important dans toutes les leishmanioses et non seulement dans les infections cutanées. Il est évident, par exemple, dans la forme leishmanoïde dermique qui succède au kala-azar et qui apparaît chez les malades indiens de 1 à 3 années après la guérison d'une infection viscérale. La réponse cellulaire de l'hôte à l'infection (principalement cellules plasmatiques et lymphocytes) détermine le taux de guérison et d'immunité ultérieure, mais la souche de *Leishmania* régit le tableau de l'infection et le degré de la virulence. Les formes

viscérales et mucocutanées sont des échecs de la réponse dermique, mais qui sont liés aux caractéristiques envahissantes propres des pathogènes en cause, peut-être modifiées par certains vecteurs ou hôtes-réservoirs. Notre compréhension de cet équilibre ou, bien trop souvent, de ce déséquilibre, dépend de la mise au point d'une méthode sûre (ou de l'adaptation de l'un des procédés modernes que l'on perfectionne actuellement au cours d'autres études) qui permette de mesurer la réponse immunitaire cutanée cellulaire de l'hôte à la présence de *Leishmania*.

## REFERENCES

- Actor, P. (1960) *Exp. Parasit.*, **10**, 1
- Adler, S. (1961) *Bull. Res. Council Israel*, **9E**, 166
- Adler, S. (1963) *Israel J. exp. Med.*, **11**, 31
- Adler, S. (1964) *Advanc. Parasit.*, **2**, 35
- Adler, S. & Nelken, D. (1965) *Trans. roy. Soc. trop. Med. Hyg.*, **59**, 59
- Adler, S. et al. (1966) *Trans. roy. Soc. trop. Med. Hyg.*, **60**, 380
- Ansari, N. (1947) *Arch. Inst. Hessarek*, **5**, 78
- Ansari, N. & Faghih, M. (1953) *Ann. Parasit. hum. comp.*, **28**, 241
- Armstrong, T. G. (1945) *Brit. med. J.*, **2**, 918
- Bray, R. S. & Rahim, G. A. F. (1969) *Trans. roy. Soc. trop. Med. Hyg.*, **63**, 378
- Cahill, K. M. (1964) *Amer. J. trop. Med. Hyg.*, **13**, 794
- Davydov, I. A. (1963) *Regional Parasit. Turkmen. SSR, Ashkhabad.*, **3**, 99
- Demina, N. A. (1962) [*The study of immunity in leishmaniasis*]. In: [*Proceedings of a Conference on Leishmaniasis and Sandfly Fever, Summaries*], Moscow, p. 94
- Demina, N. A. (1964) In: Moškovskij, S. D., ed. [*Problems of medical parasitology and prevention of infectious diseases*], Moscow, p. 303
- Gitel'zon, I. I. (1933) [*Cutaneous leishmaniasis (Pendeš sore)*]. Dissertation for the title of docent, Ashkhabad
- Hoogstraal, H. & Heyneman, D. (1969) *Amer. J. trop. Med. Hyg.*, **18**, (Suppl.), p. 1091
- Koževnikov, P. V. (1941) [*Cutaneous leishmaniasis in Turkmenia and its control*], Ashkhabad, p. 169
- Koževnikov, P. V., Dobrotvorskaja, N. V. & Latyšev, N. I. (1947) M. Medgiz [*The study of cutaneous leishmaniasis*], Moscow, 371 pp.
- Krjukova, A. P. (1941) [*Experimental cutaneous leishmaniasis in wild rodents of Turkmenia.*] In: [*Problems of cutaneous leishmaniasis*], p. 303
- Lainson, R. & Bray, R. S. (1966) *Trans. roy. Soc. trop. Med. Hyg.*, **60**, 526
- Lainson, R. & Shaw, J. J. (1966) *Trans. roy. Soc. trop. Med. Hyg.*, **60**, 533
- Manson-Bahr, P. E. C. (1961) *Trans. roy. Soc. trop. Med. Hyg.*, **55**, 550
- Manson-Bahr, P. E. C. (1963) *Active immunization in leishmaniasis*. In: Garnham, P. C. C., Pierce, A. E. & Roitt, I., ed., *Immunity to Protozoa*, Oxford, Blackwell, p. 246
- Marcinovskij, E. I. & Ščurenkova, A. I. (1924) *Russk. Ž. trop. Med. (Mosk.)*, **2**, 17
- Moškovskij, Š. D. (1937) *Med. Parazit. (Mosk.)*, **6**, 291
- Napier, L. E. (1924) *Ind. med. Gaz.*, **59**, 492
- Neal, R. (1964) *Ann. trop. Med. Parasit.*, **58**, 420
- Ni, G. V. (1967) [In: *Papers of the First Inter-Republic Conference of Middle-Asian Republics and Kazakhstan on the Problem of Basic Parasitic Diseases*], Tashkent, p. 33
- Noguchi, H. (1924) *Action of certain biological, chemical, and physical agents upon culture of Leishmania: some observations on plant and insect herpetomonads*. In: *Proceedings of the International Conference on Health Problems in Tropical America*, p. 455
- Pessôa, S. B. (1941) *Arch. Hig. (S. Paulo)*, **26**, 41
- Prata, A. R. (1957) *Hospital (Rio de J.)*, **51**, 571
- Rodjakin, N. F. (1957) [*Questions of immunity and specific prophylaxis to Borovsky's disease (cutaneous leishmaniasis)*], Turkmen. Min. Health, Derm. & Venereol. Inst. Sci. Res., *Turkmenia SSR*, 242 pp.
- Rodjakin, N. F. (1961) *Trans. Turkmen. Inst. Skin Venereal. Dis.*, **6**, 30
- Rodjakin, N. F. & Khanmamedov, N. M. (1967) In: [*Papers of the First Inter-Republic Conference of Middle Asian Republics and Kazakhstan on the Problem of Basic Parasitic Diseases*], Tashkent, pp. 39
- Saf'janova, V. M. (1966) *Med. Parazit. (Mosk.)*, **35**, 686
- Saf'janova, V. M. (1967) In: [*Papers of the First Inter-Republic Conference of Middle Asian Republics and Kazakhstan on the Problem of Basic Parasitic Diseases*], Tashkent, pp. 41
- Schneider, C. R. & Hertig, M. (1966) *Exp. Parasit.*, **18**, 25
- Sen Gupta, P. C. (1962) *Rev. Inst. Med. trop. S. Paulo*, **4**, 130
- Šišljaeva-Matova, Z. S. et al. (1966) *Med. Parazit. (Mosk.)*, **35**, 266

- Southgate, B. A. & Manson-Bahr, P. E. C. (1967) *J. trop. Med. Hyg.*, **70**, 29
- Southgate, B. A. & Oriedo, B. V. E. (1967) *J. trop. Med. Hyg.*, **70**, 1
- Stauber, L. A. (1955) *Leishmaniasis in the hamster*. In: Cole, W. H., ed., *Some physiological aspects and consequences of parasitism*, New Brunswick, New Jersey, Rutgers University Press, p. 76
- Stauber, L. A. (1958) *Rice Inst. Pamph.*, **45**, 80
- Strangeways-Dixon, J. & Lainson, R. (1962) *Brit. med. J.*, **1**, 297
- Šubarova, A. S. (1957) [*Trans. Turkmen. Inst. Skin Venereal Dis.*], **5**, 140
- Vasina, S. G., Demina, N. A. & Glazunova, Z. I. (1965) *Med. Parazit. (Mosk.)*, **34**, 708
- Vavilova, M. P. (1960) *Med. Parazit. (Mosk.)*, **29**, 660
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