

Cell-mediated immunity during syphilis

A review

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SUMMARY Evidence is presented which reinforces the complexity of the host-parasite interaction during the course of syphilis. Infection with *Treponema pallidum* evokes a complicated antibody response and an assortment of cell-mediated immune reactions in the host. It appears that humoral immunity plays a minor role towards the complete elimination of syphilitic infection while the cellular limb of the immune response may be an important host defence mechanism. Information now available indicates that a state of anergy, or immunosuppression, exists in the early stages of human and experimental rabbit syphilis based upon negative skin reactions to *T. pallidum* antigen(s), the abnormal histological appearance of lymphoid organs, and impaired *in vitro* lymphocyte reactivity. It is also evident that in the later stages of the disease cellular immunity becomes activated as delayed type skin reactions can normally be elicited in tertiary syphilitics and lymphocyte behaviour in cell culture appears normal. Several mechanisms have been invoked to explain the delay in an effective immune response against syphilitic infection and the duration of the disease: (1) a capsule-like substance on the outer surface of virulent *T. pallidum* may act as a barrier against treponemidal antibody; (2) this material and other biological properties of virulent treponemes could enable spirochaetes to escape being engulfed by macrophages and other phagocytic cells; (3) antigenic competition among different treponemal antigens causing partial tolerance; (4) *T. pallidum* infection may bring about the elaboration of immunosuppressive substances of host or treponemal origin which inhibit the proper function of lymphocytes, macrophages, and other cell types.

Introduction

Since Schaudinn and Hoffman (1905) identified *Treponema pallidum* as the causative agent of syphilis, the immunological phenomena associated with the disease have intrigued researchers and clinicians. During the past seven decades attempts to grow virulent *T. pallidum* on artificial media and to develop a successful vaccine have proved fruitless although there have been several unconfirmed reports claiming successful *in vitro* cultivation of the pathogenic spirochaete (Noguchi, 1911; Jones *et al.*, 1976). Without cultivable virulent treponemes, pure organisms devoid of host tissues are not available in

the quantities needed for biological and immunological studies. Consequently the nature of the basic immunological mechanisms elicited in the diseased host by the fastidious spirochaete or its fractions remains an enigma. Despite these obstacles considerable information has accumulated concerning antibody formation, cell-mediated immune reactions, and other aspects of host resistance to syphilitic infection (Turner and Hollander, 1957; Cannefax, 1965; Wigfield, 1965).

ANTIBODY FORMATION

Infection with *T. pallidum* stimulates the host defence mechanisms and provokes a complex antibody response. Data strongly indicate that at least two varieties of antibody are produced as syphilis progresses from early to late stages. One type (Wassermann or reagin) is non-specific and reacts primarily with tissue extracts composed of lipids; the other type (immobilising, agglutinating, fluorescent, treponemal) is specific since it reacts with some,

This work was supported by PHS grants 1-R01-GM-21313 from the National Institute of General Medical Sciences and AI-11283 from the National Institute of Allergy and Infectious Diseases

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Received for publication 17 October 1977

as yet, unidentified component(s) of the treponeme. It has been reported that one or more of these antibodies are treponemocidal and are supposedly protective (Nelson and Meyer, 1949). However the role of humoral immunity has not been delineated as some individuals with syphilis progress through primary, secondary, and tertiary stages of infection despite the formation of these various antibodies. Furthermore attempts to demonstrate a humoral mechanism by *in vivo* passive immunisation experiments with presumably immune syphilitic sera from *T. pallidum* infected rabbits have had limited success (Turner *et al.*, 1973; Weiser *et al.*, 1976) thereby suggesting that antitreponemal antibodies provide, at best, partial protection against syphilitic infection.

CELL-MEDIATED IMMUNITY

The part played by cell-mediated immunity (CMI) in the host defence against *T. pallidum* infection has also not been determined. Before the work of the past 10 years on the possible role of cellular immunity in syphilis, very little information was available on this aspect of the host response. In the early part of this century Noguchi (1911, 1912) was able to obtain a skin reaction using an extract of pure cultures of *T. pallidum* which was termed luetin. He found that an intradermal injection of luetin after repeated injections of either living or dead *T. pallidum* into rabbit testes elicited a local inflammatory reaction, and yet there was no reaction in rabbits suffering from acute orchitis. He also demonstrated similar skin reactions (sometimes severe) in tertiary stages of human syphilis, while those patients in the primary and secondary stages exhibited occasional reactions or mild ones. In contrast to Noguchi's findings, Rich *et al.* (1933) were unable to demonstrate any allergic type reactions in previously infected rabbits when reinoculated with virulent *T. pallidum* at periods ranging from 28 to 482 days after the initial immunising infection. These authors concluded that allergic inflammation may not be associated with any acquired immunity in experimental rabbit syphilis. In another report, Marshak and Rothman (1951) found that only patients who had acquired gummatous or congenital syphilis gave strong tuberculin-type reactions to a purified suspension of *T. pallidum* prepared from infected rabbit testes, while six of seven individuals with secondary acquired syphilis yielded negative reactions. Similarly only 60% of rabbits infected for a two- to 12-month period gave a positive skin reaction to the treponemal extract. No correlation existed between the duration of the infection and the degree of skin reaction. An analogous study conducted by a French group (Thivolet *et al.*, 1953) showed that delayed-type skin

reactions to dead *T. pallidum* were rarely elicited in patients with primary and secondary syphilis, whereas a large percentage of tertiary syphilitics reacted positively to intradermal injection of this treponemal extract. Other experiments performed by Laird and Thorburn (1966) showed that 67% of their patients with tertiary syphilis gave delayed-type hypersensitivity reaction to an extract of *T. pallidum*, the luotest, which occurred after repeated challenge. The unresponsiveness in early syphilis seems to be specific to *T. pallidum* antigens since skin reactions to several non-treponemal antigens—such as, mumps, old tuberculin, and varidase—were reported in patients at various stages of syphilis (Wright and Grimble, 1974).

Some investigations have been performed relating the histological appearance of lymphoid tissues with the presence of *T. pallidum* infection. In a study in which lymph nodes of 20 patients with early syphilis were removed, Turner and Wright (1973) were able to show a depletion of lymphocytes in the paracortical areas of these tissues. Numerous treponemes were found in these areas using the silver impregnation technique. Levene *et al.* (1971) while conducting a study of infants dying from congenital syphilis discovered a diminution of lymphocytes in the area around the central arteriole of the spleen. Comparable results were obtained by Fenstein *et al.* (1967) who infected neonatal rabbits with *T. pallidum*. These infected animals exhibited a runting syndrome in which death usually occurred within three months. Depletion of lymphocytes in the white pulp areas of the spleens of these rabbits was found although treponemal invasion of this organ was not observed. The paracortical areas of lymph nodes and the area around the central splenic artery are thymus-dependent areas involved in mediating cellular immune reactions of the host, and defective CMI is associated with lymphocyte depletion in these areas (Parrott *et al.*, 1966). From these studies on the structural appearance of lymphoid tissue it is apparent that thymus-dependent (T-cell) areas are adversely affected during syphilitic infection.

Another parameter used to examine the cellular mechanisms involved in *T. pallidum* infection has been the attempt by several investigators to activate the reticuloendothelial system by inoculating rabbits with BCG* organisms and then monitoring what effect this treatment had on the course of experimental disease (Graves and Johnson, 1975; Schell *et al.*, 1975b). In these studies prior injection with BCG was unsuccessful in preventing the develop-

*BCG is a viable attenuated strain of *Mycobacterium bovis* which in the form of a vaccine has been able to confer protection against mycobacterial infection and to stimulate non-specifically CMI in certain experimental models.

ment of syphilitic lesions in rabbits subsequently inoculated with virulent *T. pallidum*. Although there was a delay in the onset of lesions when immune treponemal serum was administered simultaneously with BCG, no evidence was found suggesting a synergistic protective effect between the activated macrophages and the antitreponemal antibodies. Despite these results whereby enhanced resistance to treponemal infection owing to presumably armed macrophages was not detected in BCG-infected animals, Schell and Musher (1974) have presented indirect evidence favouring the suggestion that infection with *T. pallidum* stimulates CMI. Basically they followed the model established by Blanden *et al.* (1969) in which infection by one organism confers resistance to infection with an antigenically unrelated organism. Rabbits were infected first with virulent *T. pallidum* and then challenged with *Listeria monocytogenes*. Resistance to *Listeria* infection was then evaluated weekly by measuring the number of organisms found in the spleens and livers of the infected animals. Enhanced ability to suppress the growth of *Listeria* was detected in the rabbits' livers between the third and sixth week after intravenous infection with virulent *T. pallidum*. However there was no indication that treponemes were engulfed by these activated phagocytic cells. Additionally, in a manner similar to the adoptive transfer model of Mackaness and Hill (1969), spleen cells from syphilitic rabbits together with *T. pallidum* were transferred to normal rabbits (Schell *et al.*, 1975a). A significant degree of resistance to subsequent challenge inoculation with *Listeria* was produced in the recipient animals. The results were based on the number of *Listeria* in the livers and spleens of these rabbits. When the spleen cells from the infected donor rabbits were first reacted with a highly specific antirabbit thymus serum and complement, Schell *et al.* (1975a) showed that transfer of immunity was abrogated indicating that thymus-dependent immunity was responsible for conferring resistance to *Listeria* infection. In a similar fashion Metzger and Smogor (1975) demonstrated partial immunity to *T. pallidum* infection by adoptive transfer of lymph node lymphocytes from syphilitic rabbits to normal ones. These investigators showed that a reduced number or a delayed incubation or absence of lesions occurred in the recipient animals after intradermal challenge with virulent *T. pallidum*. However since outbred animals were used in these experiments partial protection could have resulted from non-specific sensitisation occurring when allogeneic cells present in the putative immunocompetent lymphocyte population were transferred from infected to normal rabbits.

In vitro tests for CMI, notably lymphocyte trans-

formation and cell migration inhibition, have been used to monitor the cellular immune capability of man and animals to various diseases (Kantor, 1975; Rocklin, 1976) including syphilis (Badanoiu *et al.*, 1969; Levene *et al.*, 1969; Janot *et al.*, 1971; Fulford and Brostoff, 1972; Musher *et al.*, 1974; Friedman and Turk, 1975; Musher *et al.*, 1975; Wicher and Wicher, 1975; From *et al.*, 1976; Pavia *et al.*, 1976, 1977a). In the lymphocyte transformation assay lymphoid cells usually derived from peripheral blood are cultured with a suitable stimulant, especially a mitogen—such as, phytohaemagglutinin (PHA), or concanavalin A (Con A), or a specific test antigen—in this case a treponemal preparation. The mitogen non-specifically induces a large number of cells to enlarge, proliferate, and undergo DNA synthesis whereas a narrower range of cells experience similar changes in a specific response to the antigen. The degree of blastogenesis is measured either by microscopical examination of the blast cells or more accurately by incorporating a radioactively labelled DNA precursor into newly synthesised DNA. The amount of label incorporated and therefore the rate of DNA synthesis is determined usually by scintillation counting which gives a quantitative assessment of lymphocyte responsiveness and presumably functional capacity. The results of this test using lymphocytes from syphilitic patients have so far been inconsistent. Several European investigators have been able to transform cells from individuals with syphilis. Badanoiu *et al.* (1969) showed that positive lymphocyte responses to PHA and an antigen composed of *T. pallidum* (Nichols strain) can regularly be elicited from patients with syphilis at various stages of infection. In another study, Janot *et al.* (1971) using a saline suspension of the Nichols strain of *T. pallidum* as stimulant, demonstrated increased numbers of lymphoblastoid-like cells based upon the morphology of the lymphocyte populations in four of six patients with primary, nine of 11 with secondary, five of six with congenital syphilis, as well as all of 18 with cardiovascular or neurosyphilis, 30 with latent, and 12 patients with treated syphilis. Likewise Friedman and Turk (1975) reported elevated blastogenic responses to a *T. pallidum* preparation in patients with seropositive primary infection and those in the papular stage of secondary syphilis, although patients in the macular stage of disease gave poor responses. In addition individuals undergoing treatment with drugs exhibited marked lymphocyte reactivity to the *T. pallidum* antigen. Data contrary to these findings have been provided by other investigators. Levene *et al.* (1969) demonstrated that patients with primary and secondary syphilis had an impaired lymphocyte response to PHA. At the

same time plasma from patients with secondary syphilis was found to cause reduced levels of blastogenesis of normal lymphocytes. Other workers (Kantor, 1975; From *et al.*, 1976) have shown that serum or plasma from diseased patients hampered the PHA or Con A induced response of normal lymphocytes. In two studies Musher *et al.* (1974, 1975) showed that primary and secondary syphilitic exhibited a suppressed *in vitro* lymphocyte response to treponemal antigens derived from *Treponema refringens*, *Treponema phagedenis* biotype Reiter, and *T. pallidum*, and to non-treponemal antigens such as *Candida* and trichophytins. However these patients demonstrated a normal lymphocyte response to PHA, pokeweed mitogen, and streptolysin O. Moreover syphilitic serum possessed no *in vitro* inhibitory activity. Another *in vitro* correlate of CMI, the leucocyte migration inhibition test, has been used in several studies for examining the cellular response of syphilitic patients (Fulford and Brostoff, 1972; From *et al.*, 1976) and of experimentally infected rabbits (Wicher and Wicher, 1975; Metzger *et al.*, 1977). In this particular assay migration inhibition is measured directly using peripheral blood leucocytes or indirectly using sensitised lymphocytes and guinea-pig macrophages and placing them either in capillary tubes or in agar in the presence or absence of appropriate antigen. Cell migration is measured between 24 and 48 hours later and the relative areas of migration are determined, usually by planimetry, and the amount of inhibition of cell migration is calculated. Inhibition of cell migration is closely associated with the presence of *in vivo* cellular hypersensitivity of the host to that antigen (Rocklin, 1976). Using this assay Fulford and Brostoff (1972) assessed the reaction of 47 patients who were at various stages of syphilis to a commercial preparation of Reiter protein as antigen. They found that in 19 cases of primary syphilis, patients exhibited stimulation of migration; 24 patients with either secondary or latent syphilis showed neither stimulation nor inhibition. Only in those individuals with late active syphilis was there clear evidence of inhibition of migration which could be interpreted as a manifestation of CMI to Reiter protein. In a similar report (From *et al.*, 1976), it was demonstrated that increased cellular reactivity evaluated by migration inhibition in response to a *T. pallidum* antigen became evident after syphilitic patients received drug treatment. Another study (Wicher and Wicher, 1975) investigated the cellular response of syphilitic rabbits by measuring leucocyte migration in agarose using a *T. pallidum* antigen, a commercial Reiter antigen, and cardiolipin reagent as stimulants. A bimodal response was demonstrated by cells in the presence of each of the

commercial preparations and a low concentration of *T. pallidum* antigen. During the first four weeks of syphilitic infection, stimulation of leucocyte migration occurred (indicative of weak CMI), while after this period inhibition of migration was observed (indicative of strong CMI). When cells were exposed to a higher concentration of *T. pallidum* antigen inhibition of migration was seen from the first week of infection onwards. Similar data have been given by Metzger *et al.* (1977) in a long-term study whereby the macrophage migration inhibition test was used for evaluating the cellular response during experimental disease. It was demonstrated that from one month after infection lymphocytes from *T. pallidum* infected rabbits exposed *in vitro* to a sonicated treponemal suspension significantly inhibited the migration of guinea-pig macrophages. The inhibitory effect was maintained for at least two years of infection except for a transient decrease in inhibition activity between the fourth and sixth month.

In our series of experiments we investigated the CMI response of rabbits infected with *T. pallidum* by employing the lymphocyte transformation assay (Pavia *et al.*, 1976, 1977a) and the macrophage migration inhibition test (Pavia *et al.*, 1977b). Although the rabbit may not be suitable for certain immunological studies we chose this animal for our investigation as experimental infection in the rabbit has so far been the model of choice in attempting to elucidate the nature of immunological phenomena associated with the disease and for making comparisons with human syphilis. Furthermore a local lesion can be readily produced at the site of inoculation of virulent *T. pallidum* and the clinical course of the disease can easily be monitored as the infection progresses from early to late stages. The study consisted of collecting blood weekly from *T. pallidum* infected rabbits and culturing their peripheral blood lymphocytes with various mitogens and Reiter treponemal antigen. During the first three or four weeks of infection the transformation of lymphocytes from infected rabbits by Con A, PHA, and pokeweed mitogen was suppressed when compared with the lymphocyte response of non-infected control rabbits. In addition, serum from the donor rabbit significantly reduced the Con A-induced response of autologous lymphocytes. After four weeks, normal levels of blastogenesis were usually observed. When lymphocytes from these same infected animals were exposed *in vitro* to antirabbit immunoglobulin and to a Reiter treponemal preparation a different pattern emerged. Throughout the duration of infection normal levels of blast transformation were obtained in response to these stimulants.

In the next sequence of experiments peritoneal

exudate cells from *T. pallidum* infected rabbits were collected every two weeks and examined for migration inhibition activity in the presence of *T. phagedenis* biotype Reiter. Between five and 15 weeks after infection there was a significant inhibition of macrophage migration in response to three different concentrations of the Reiter treponeme. Before this period little or no inhibition had been evident. The inhibitory effect manifested in these animals could be interpreted as a response by sensitised cells to antigenic determinants of the Reiter treponeme which are shared with the virulent Nichols *T. pallidum* (Meyer and Hunter, 1967).

Our findings suggested an interrelationship between the appearance and regression of symptomatic infection and the *in vitro* response to T-cell mitogens (Con A, PHA, pokeweed mitogen) in the transformation test and *T. phagedenis* biotype Reiter in the migration inhibition assay. During the first few weeks of infection in which widespread dissemination of organisms occurred and severe ulcerative lesions developed the rabbits manifested poor lymphocyte reactivity, whereas during remission when lesions were subsiding (four to six weeks after infection) blastogenesis and migration inhibition markedly increased. This may reflect heightened T-cell reactivity at this stage of infection. Furthermore the normal blastogenic response to anti-immunoglobulins (putative B-cell mitogens) may correlate with the initiation of antibody synthesis to treponemal antigens and thus reflect an intact humoral expression of immunity in syphilis.

Recently we have reinforced previous data describing the apparent selectivity of the lymphocyte response during experimental *T. pallidum* infection by separating rabbit lymphocytes into T- and B-cell populations and monitoring their *in vitro* response to appropriate mitogens and *T. pallidum* antigen(s) (Pavia *et al.*, 1977c). It was clear that Con A and PHA-induced proliferation of the purified T cells was markedly reduced during early infection while the response to classical B-cell mitogens was normal. In striking contrast, the same enriched T-cell population from infected animals was stimulated by *T. pallidum* antigen(s). However autologous syphilitic serum significantly diminished the *in vitro* stimulatory effects of *T. pallidum* antigen(s) on the enriched T cells. A relevant conclusion from these studies is that during early disease the T cells that exist in the infected host respond poorly to certain mitogens while these cells, or a subpopulation of them, are readily stimulated by *T. pallidum*. This latter phenomenon, in turn, is subject to the regulatory effects of syphilitic serum which could adversely affect *in vivo* as well as *in vitro* lymphocyte reactivity.

Proposed mechanisms of immunity in syphilis

Limited information is available concerning the nature of immune mechanisms during *T. pallidum* infection. Although a role for humoral immunity was implicated when it was apparently demonstrated that protective antibodies directed against *T. pallidum* were present in serum taken from infected patients and rabbits (Turner, 1939; Turner *et al.*, 1948), it was later suggested (Turner and Hollander, 1957) that the phenomena probably represented an *in vitro* effect rather than a direct *in vivo* consequence. Also the general pattern of evidence indicates little correlation between the level of antibody and the degree of immunity to challenge of previously infected or immunised rabbits (Magnuson *et al.*, 1951; Metzger *et al.*, 1969; Miller, 1973) and it has also been demonstrated that complete protection against experimental *T. pallidum* infection can be accomplished without the immediate presence of circulating antibody (Miller, 1973).

A relationship may exist between the inherent properties of the infectious treponeme and the role of immune mechanisms in syphilis. Factors intrinsic to the treponeme may enable it to circumvent or block immunological attack. It has recently been shown (Zeigler *et al.*, 1976) that the virulent Nichols strain of *T. pallidum* possesses an extracellular layer. This outer coat was observed *in vivo* and *in vitro* after the organisms were exposed to ruthenium red. Before this report, only presumptive evidence for this structural component existed (Metzger *et al.*, 1961; Christiansen, 1963). Some researchers (Metzger *et al.*, 1961; Metzger, 1962; Miller, 1967) have advanced the hypothesis that acquired resistance may depend on the association between treponemocidal antibody and this outer capsule-like material. It has been suggested that treponemocidal antibody can exert an inhibitory effect on the organisms once the outer capsule has been removed or somehow been stripped off. What mechanisms are involved in the disappearance of this outer coat are not known but the relatively slow development of immunity to *T. pallidum* infection may be directly related to the poor antigenic nature of this outer material and its half life *in vivo*.

Since the role played by antibody towards the eradication of syphilitic infection is not clear it may be necessary to invoke cell-mediated immune phenomena as important factors in the infectious process. It has already been demonstrated (Turner and Hollander, 1957) that vaccines consisting of dead or purified antigen preparations of *T. pallidum* have generally been unsuccessful in providing effective protection against treponemal infection despite the formation of an abundance of antibody. However

complete resistance to *T. pallidum* infection has been produced by prior injections of motile virulent treponemes attenuated by γ -irradiation (Miller, 1973). Elberg (1973) showed that efficient induction of CMI in certain microbial infections requires living vaccines, whereas killed vaccines or purified bacterial antigens are less efficient forms of immunoprophylaxis. Taken together, these facts suggest that protection against *T. pallidum* infection may depend on a specifically activated cellular immune system. In addition it is becoming apparent that the cellular limb of the immune response is defective in the highly infectious early stages of syphilis and is mobilised at other stages. Antigenic competition has been considered a possible candidate underlying the hyporeactive state in early disease (Wright and Grimble, 1974). If *T. pallidum* possesses multiple antigenic determinants (and this seems likely) one antigen could selectively compete and interfere with the response to other antigens, thereby creating a condition of partial or split tolerance. Another conceivable explanation is that this transient period of immunosuppression may be the result of a modification of T-cell or macrophage function by blood factors or by other cellular components of host (immune complexes, suppressor substances) or treponemal (outer envelope, metabolic byproduct) origin generated during syphilitic infection. Effector lymphocytes may be suppressed by a 'blocking factor' analogous to that described in tumour immunity and in certain experimental systems (Hellstrom and Hellstrom, 1970) so that they are unable to differentiate or carry out effector function. In fact, inhibitory plasma or serum products have been demonstrated in syphilitic patients (Levene *et al.*, 1969; Kantor, 1975) and in *T. pallidum* infected rabbits (Pavia *et al.*, 1976; 1977c). In human syphilitic glomerulonephritis deposits of treponemal antigen-antitreponemal antibody complexes have been shown to contribute to tissue injury (Gambol and Reardon, 1975). Although suppressor cells have not been studied in syphilis they could be an important consequence of *T. pallidum* infection especially in the light of the recent finding of suppressor thymus-derived lymphocytes in patients infected with a variety of fungal organisms (Stobo *et al.*, 1976). In our recent report (Pavia *et al.*, 1977c) on experimental *T. pallidum* infection in rabbits the existence of suppressor-like function is suggested by a delay in the response to *T. pallidum* antigen(s) of unfractionated lymphocytes in contrast to purified cells. Suppressor cells could inactivate immune lymphocytes which arise at foci of treponemal infection. A regulatory effect exerted on immunocompetent cells by any of the preceding conditions could also block the release of soluble mediators

collectively known as lymphokines, one of which, migration inhibitory factor, encourages circulating blood monocytes to localise at sites of microbial invasion. The inability of effector cells such as lymphocytes and macrophages to be recruited to specific tissues in the diseased host and/or the capacity of *T. pallidum* to resist phagocytosis due to the organism's innate biological properties may delay the early destruction of virulent treponemes and contribute to the widespread dissemination and establishment of spirochaetes in selected host sites. Then as the infection progresses to later stages a transition in the immune status of the host takes place. Normal T-lymphocyte function is restored leading to an effective cell-mediated response which could allow host defence mechanisms to participate in treponemicidal activities. From the sequence just presented it should be realised that cell-mediated phenomena in syphilis are by no means expressions of a single mechanism. Different cell categories (T-cells, B-cells, monocytes, macrophages, neutrophils) may be involved and their relative contribution to immunity may depend on the dynamic interaction of various factors induced by *T. pallidum* infection. If indeed cell-mediated immunity is an important element against syphilitic infection then investigations should be carried out in order to determine the extent of its protective role in this disease.

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