RESISTANCE ABROGATION TO MURINE HISTOPLASMOSIS INDUCED BY ANTIBODY*

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ABATIMIENTO DE LA RESISTENCIA A LA HISTOPLASMOSIS MURINA INDUCIDA POR ANTICUERPOS

RESUMEN

Se siguió el curso de la respuesta humoral y celular a través de la infección experimental histoplasmosa. Se estudió el posible papel modulador del suero inmune sobre la respuesta mediada por células desarrolladas durante la infección producida por *Histoplasma capsulatum.* Se demuestra que existe una distribución cíclica de la respuesta inmune celular y humoral en los ratones infectados, que la transferencia pasiva de anticuerpos anti-*Histoplasma* abate la sobrevida de los ratones infectados subletalmente, cuando el suero fue transferido 3 h antes de la infección. Los resultados de este modelo experimental sugieren que los anticuerpos pueden jugar un papel modulador de la respuesta celular en la histoplasmosis.

SUMMARY

The course of humoral and cellular responses were followed throughout infection in experimental histoplasmosis. A possible modulatory role of immune sera in the cell-mediated immune response, developed during the infection produced by *Histoplasma capsulatum,* was also studied. The results demonstrated that there was a cyclic distribution of cellular and humoral immune responses in infected mice and that the passive transfer of anti-*Histoplasma* antibodies abrogated the survival of mice sublethally infected, when the sera were transferred 3 h prior to infection. The results obtained from this experimental model suggest

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that antibody may play a modulating role in cell-mediated responses in histoplasmosis.

INTRODUCTION

Histoplasmosis is a fungal disease produced by *Histoplasma capsulatum,* which induces a typical intracellular host-parasite relationship. It shares with other intracellular microorganisms, the characteristic that the pathogenic mechanism by which the etiologic agent causes tissue damage during the infection is unknown (Emmons *et al.,* 1977; Rippon, 1974; Youmans *et al.,* 1980).

Throughout the illness, the immune response is characterized by the inefficiency of the host in developing an optimal response; sometimes this circumstance is manifested by hyperreactivity of the cell mediated response, commited with the pathology of the infectious process (Youmans *et al.,* 1980). Consequently, an adequate modulation of the immune mechanisms implicates its efficiency in the outcome of the disease.

It is accepted that regulatory functions of the immune system involve different mechanisms of the immune response. Among the diversity of the immunoregulatory mechanisms, humoral effectors have special interest due to the critical role that antibodies could play on the regulation of the humoral and cellular immune responses. Today, it is assumed that anti-idiotypic antibodies suppress delayed-type hypersensitivity (DTH) to haptens like p-azobenzenearsonate (Sy *et al.,* 1979), phosphoryl choline (Yamamoto and Katz, 1979), etc. However, little consideration has been given to the specific antibody as a regulatory component, although its influence on antibody production via a feedback mechanism was described many years ago (Uhr and Baumann, 1961; Uhr and Möller, 1968). The role of specific antibodies on the intracellular infection is contradictory, their participation on immune damage has been proposed in some illnesses (Youmans *et al.,* 1980) and their usefulness as a diagnostic and pronostic tool has been discussed.

Some evidence points to antibodies as regulatory elements in infectious processes (Colizzi *et al.,* 1983; Lenzini *et al.,* 1977; Ridley and Jopling, 1966; Taylor *et al.,* 1984), furthermore auto-anti-idiotypic antibodies have been shown to inhibit cells from BCG infected mice, which transferred the delayed type hipersensitivity response to purified protein derivative (PPD) in receptor mice (Colizzi *et al.,* 1983).

Different observations support the role of the antibody as a regulatory component over cellular mediated immunity in histoplasmosis: first, the relationship between high antibody titres and low cellular response which concurs with a critical outcome of the disease (Emmons *et al.,* 1977; Rippon, 1974; Youmans *et al.,* 1980); second, the inhibition of lymphocyte transformation induced by the antigen histoplasmin in the presence of anti-*Histoplasma* antibodies (Newberry *et al.,* 1968); and third, the inverse correlation between

cellular and humoral response during the course of experimental histoplasmosis (Taylor *et al.,* 1980a).

However, evidence is circumstantial and more studies are necessary to support the regulatory role of antibodies on cellular response in models of intracellular infections. The development of the cellular and humoral response using a murine *Histoplasma* infection model was examined, in attempts to elucidate the influence of antibodies on the protective immune mechanism (cell mediated response) triggered by this infection.

MATERIALS AND METHODS

Culture. H. capsulatum strain 5037, tested for its virulence was grown in yeast-phase cell at 37°C in brain heart infusion broth (BHI) (Bioxon, Mexico) supplemented with glucose 10gl^{-1} and L-cysteine 1 gl^{-1} .

Animals. Young (1 month) and adult (4.5 months) inbred BALB/c male mice were used and maintained in our laboratory with animal facilities. Mice where originally separated by age and given mouse chow (Purina de México, S.A. de C.V.) water *ad libitum.*

Histoplasma inocula. The fungus was grown in supplemented BHI for three days and harvested by centrifugation at 300 x g for 30 minutes. Organisms were then inoculated into a synthetic medium (Tewari and Kugel, 1971). They were harvested again and suspended in a balanced saline solution (BSS) at the desired concentration. All inocula were tested for viability and conversion to mycelial phase at 28°C on BHI agar slants.

Histoplasma infection. Yeast cells contained in one ml of BSS were inoculated intraperitoneally into mice. A sublethal dose for young (3.16 x 10^{7} cells ml^{-1}) and for adult (1.99 x 10^{8} cells ml^{-1}) mice was used throughout the study as determined in a previous experiment (Taylor *et al.,* 1982). Another group of animals was processed in the same manner in order to obtain only antisera.

Histoplasma inmune sera separation. Blood was collected from animals 30 days after infection. Globulins were obtained at 4°C by ammonium sulphate precipitation at 33% saturation, pH. 6.8. The precipitated globulins were centrifugated at 2500 x g, redissolved in distilled water and dialyzed against phosphate buffered saline (PBS) pH 7.2 (Heide and Schwick, 1978). Globulins from control mice were fractionated by the same method. Proteins concentration were determined according to Lowry *et al.* (1951).

Antibody determination. The antibody titres for passive serum transfer of anti-*Histoplasma* immune sera, were determined by ELISA method (Yolken, 1982) using mouse anti-gammaglobulin and anti-IgG alkaline phosphatase conjugate (Sigma). This method nas been standardized in our laboratory by previous titration of histoplasmin antigen, which had its optimal concentration at 100 ug of protein

for each well polystyrene plates.

Correlation between cellular and humoral immune response throughout infection. A total of 120 mice for each experimental group were used, using 5 animals for each point. Groups of mice aged one or 4.5 months were tested at different times. Infection with a sublethal dose of *Histoplasma* yeast cells for each age group was carried out as described above. Cellular and humoral responses were monitored every 8 day.

Cellular response was measured by delayed-type hypersensitivity (DTH), expressed by the footpad swelling test as described previously (Rifkin *et al.,* 1970; Taylor *et al.*, 1980b) using histoplasmin (30 ug protein per 0.05 ml) from a single lot, reading the test 24 h after antigen challenge. The humoral response was detected by passive hemagglutination assay (Avrameas, 1967) using sheep erythrocytes (SRBC) coated with histoplasmin at 1 mg protein ml^{-1} (Reyes Montes *et al.,* 1982). The titre recorded is the highest dilution of antiserum in which agglutination was demonstrated. Two experiments per age group were analized by statistical methods and in a third experiment, cellular response was also checked by macrophage inhibition factor (MIF) production and the humoral response was confirmed by the ELISA method.

Passive serum transfer versus percent survival to Histoplasma infection. Histoplasma immune sera or normal sera, were injected subcutaneously (300 ug of protein per 0.3 ml) 3 h prior to, or 10 days after *Histoplasma* infection. The specificity of sera was tested by ELISA. Experiments were performed by duplicate. Normal and *Histoplasma* immune sera were transferred and the percent survival was determined over 30-40 days after infection. A total of 62 mice were used per experiment. Animals death were checked by organ cultur for the fungus.

Organ cultures. Spleen, lung and liver from infected dead mice were homogenized in BSS supplemented with 50 ug ml^{-1} gentamycin. Homogenates were inoculated in duplicate into Sabouraud agar with antibiotics (Mycobiotic Bioxon de México) and BHI-agar at 26-28°C.

RESULTS

Correlation between cellular and humoral immune response throughout infection. Young and adult mice with sublethal infection, were monitored for their cellular and humoral responses every 8 days over a 3 month period. Both responses are plotted together to establish a comparison between the immune manifestations throughout the infection. Figure 1 and 2 illustrate the results for the two age groups used. The results exhibit a cyclic distribution with a displacement between each immune response. A special characteristic that can be observed is the decrease of the cellular response measured by the footpad swelling test ,

which apparently precedes the increase of antibody titres detected by hemagglutination. For young mice (Fig. 1) there was a depression in cell mediated responses at two points, days 40 and 88. While the humoral response increased at days 32, 80 and 88. With the adult mice (Fig. 2), the cellular response decreased at 24, 64 and 88 days respectively, while antibodies increased at 32 and 72 days. MIF and ELISA showed similar results to the previous tests.

Antibody determination. Antibodies to *Histoplasma* detected by the ELISA method, gave to anti-*Histoplasma* a titre of 1:1280. Normal sera were always negative to histoplasmin antigens.

Passive serum transfer versus percent survival to Histoplasma infection. Figure 3 illustrates a drastic abrogation of survival (10%) of adult mice, when anti-*Histoplasma* antibodies were transferred 3 h prior to infection, but antibody transferred 10 days postinfection did not modify the survival of the infected animals (100%). Seventy percent survival was observed in the infection controls without serum transfer. Normal sera did not interfere with the outcome of the disease, since a 66.6% survival was detected when it was transferred 3 h prior to infection.

Organs cultures. Table 1 shows the isolation of the fungus, particularly from the spleens of infected mice which were transferred with immune sera. *H. capsulatum* was isolated at days 15, 24 and 32 from all groups of transferred and infected mice, except at day 8 when the transfer of immune sera was made 3 h prior to infection.

DISCUSSION

Relationship between antibody and cell mediated response in histoplasmosis is controversial. Serum causes specific suppression in active human histoplasmosis, since response to mitogen and other fungal antigens were unaffected. Sera of patients with histoplasmosis suppressed lymphocyte transformation (LT) of healthy histoplasmin-positive reactors, while sera of healthy histoplasmin reactive persons increased histoplasmin response of patients (Kirkpatrick *et al.*, 1971; Newberry *et al.*, 1968). Alford and Goodwin (1972) have suggested that antibodies may increase or decrease LT responses depending on the ratio of antigen to antibody.

Little evidences concerning a definite role for antibodies in the process of *Histoplasma* infection adds to the concept of its diminished importance in the defense against this disease, prompting us to investigate the probable function of these molecules in the course and the outcome of this intracellular illness.

Using an experimental murine model of histoplasmosis, in which disease is characterized by typical signs of the infection in mice and the isolation of fungus from the tissue, our results support the possible participation of antibodies in the

immune modulation mechanisms of the cell mediated response. The first series of assays demonstrate a correlation between cellular and humoral responses during the infectious process in young and adult mice, and provide evidence for a cyclic distribution in both immune responses with a special characteristic of displacement between them (Figs. 1 and 2). These circumstances, whereby the increase of one response precedes the decrease of another response, suggests the existence of a modulation mechanism. Furthermore, in some experiments a good correlation among hemagglutination and ELISA titres was detected. Using the MIF method to test the cellular response, we also observed the same phenomenon.

It is important to note that these results are only observed with a sublethal infection, because in other circumstances (severe acute phase) the drastic resolution of the illness abbreviates the animals survival. In order to determine if sera were capable of changing the cellular defense, passive serum transfer was performed. The Next experiment suggest an interference of antibodies in the efficiency of the cellular defense mechanism in histoplasmosis. The effect of anti-*Histoplasma* antibodies depends on the time of transference in accordance with the moment of infection. In animals, the disease has a drastic outcome if anti-*Histoplasma* antibodies are injected 3 h prior to infection, however, if the transference occurs 10 days after infection there is no influence in the percent survival of the infected animals (Fig. 3). It is possible that the behavior of each group of transferred mice involved different mechanisms. The serum transfer at 3 h prior infection, could interfere with the afferent phase of the immune reaction and the macrophage could be the target cell for the sera effect. So, the ability to develop a complete and functional protective cellular response by sublethally infected animals was abrogated only by the immune sera transfer 3 h prior infection in all experiments.

The role of specific antibodies in intracellular infections is a very interesting problem. For example it would appear, that an antibody, when present before *Coxiella burnetti* infection accelerates the initial interactions of the inductible phase of the cellular immune response (Humphres and Hinrichs, 1981), however it is difficult to characterize the exact effect of antibodies under these circumstances.

Regulatory mechanisms have many variations in their specificity, one of the most interesting aspects involves immune complex and anti-idiotypic antibodes. It is possible that specific antibodies or some immunoglobulins produced during the infectious process act like anti-idiotypic antibodies, or induce anti-idiotype formation (Binz and Wigzell, 1975; Dressman and Kennedy, 1985). They could react with antigen receptors present on the lymphocytes membrane, which share analogous epitopes with the idiotypes present in the anti-*Histoplasma* antibodies, therefore they could have anti-receptor activity (Binz and Wigzell, 1975). It is possible also that antigen-antibody complex are efficient to induce anti-idiotype antibodies and some reports suggest that they activate suppressor cells (Sy *et al.*, 1979).

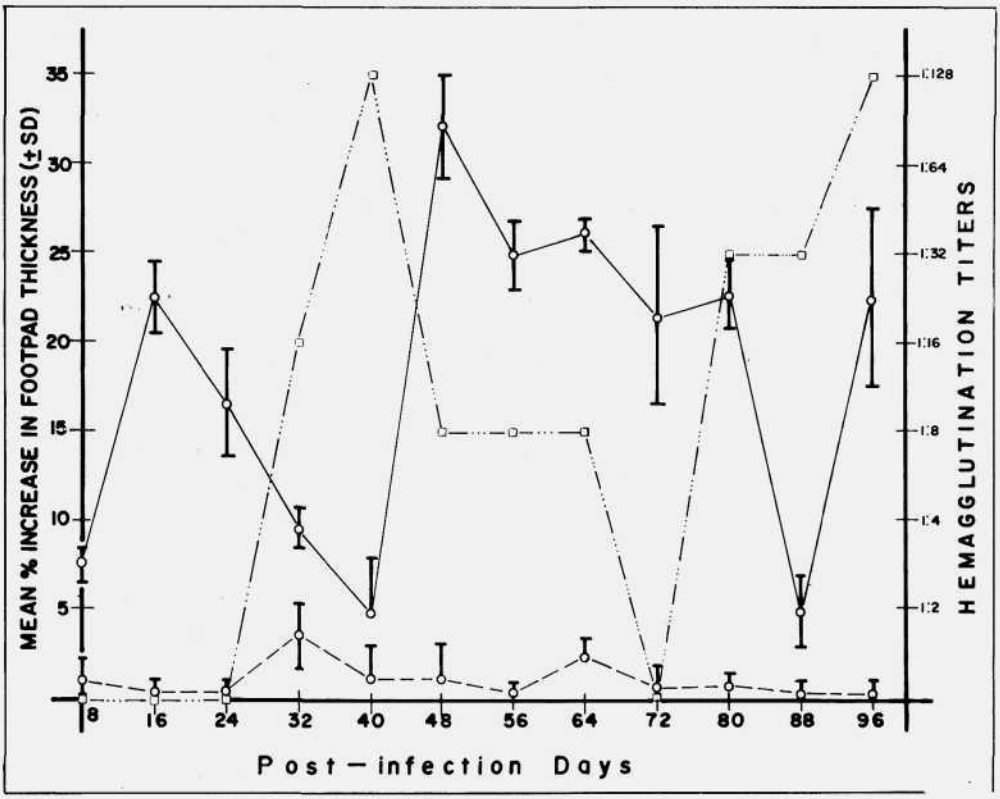

Fig. 1. CORRELATION BETWEEN CELLULAR AND HUMORAL IMMUNE RESPONSE IN YOUNG MICE DURING THE *Histoplasma* INFECTION. Footpad swelling test vs. hemagglutination. Syngeneic male BALB/c mice were used.
0—0 mean percent of increase in footpad thickness in infected mice using histoplasmin antigen (30 ug protein per 0.05 ml) minus the histoplasmin antigen value in normal mice: 0···0 mean percent of increase in footpad thickness in infected mice using control with saline solution minus the value in normal mice; □—·····—□ hemagglutination: test performed by the glutaraldehyde method. Histoplasmin at 1 mg protein ml^{-1} was used as antigen.
± SD=Standard Deviation

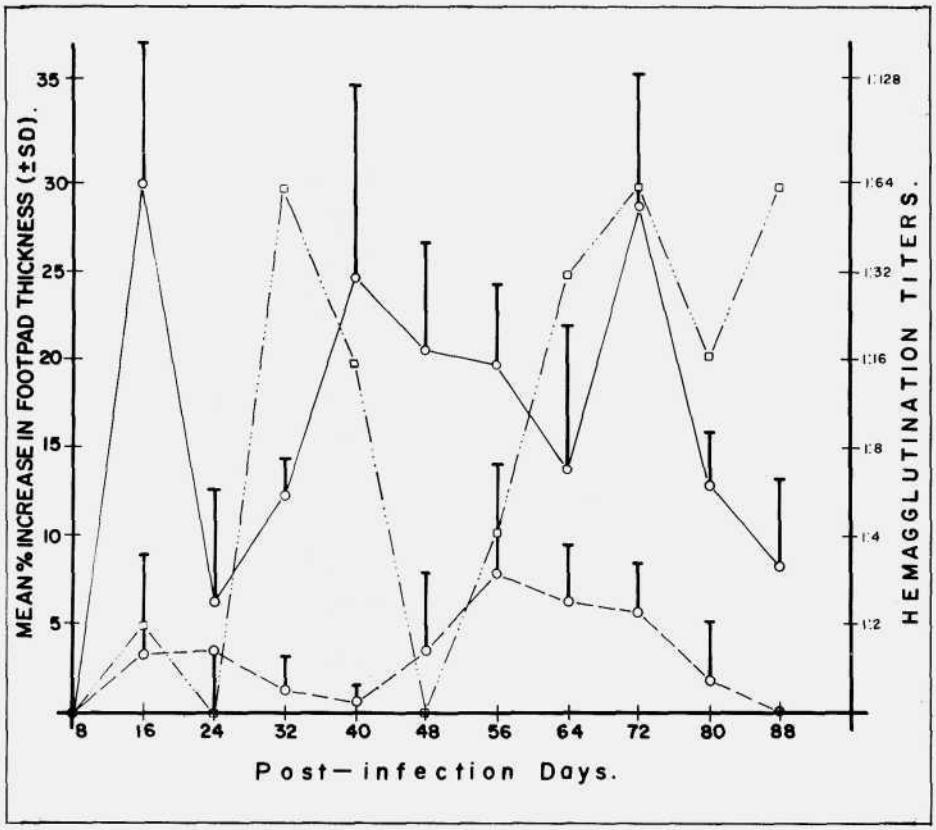

FIG. 2. CORRELATION BETWEEN CELLULAR AND HUMORAL IMMUNE RESPONSE IN ADULTS MICE DURING THE *Histoplasma* INFECTION. Footpad swelling test vs hemagglutination. Syngeneic male BALB/c mice were used. All experiment conditions and symbols are the same as figure 1.

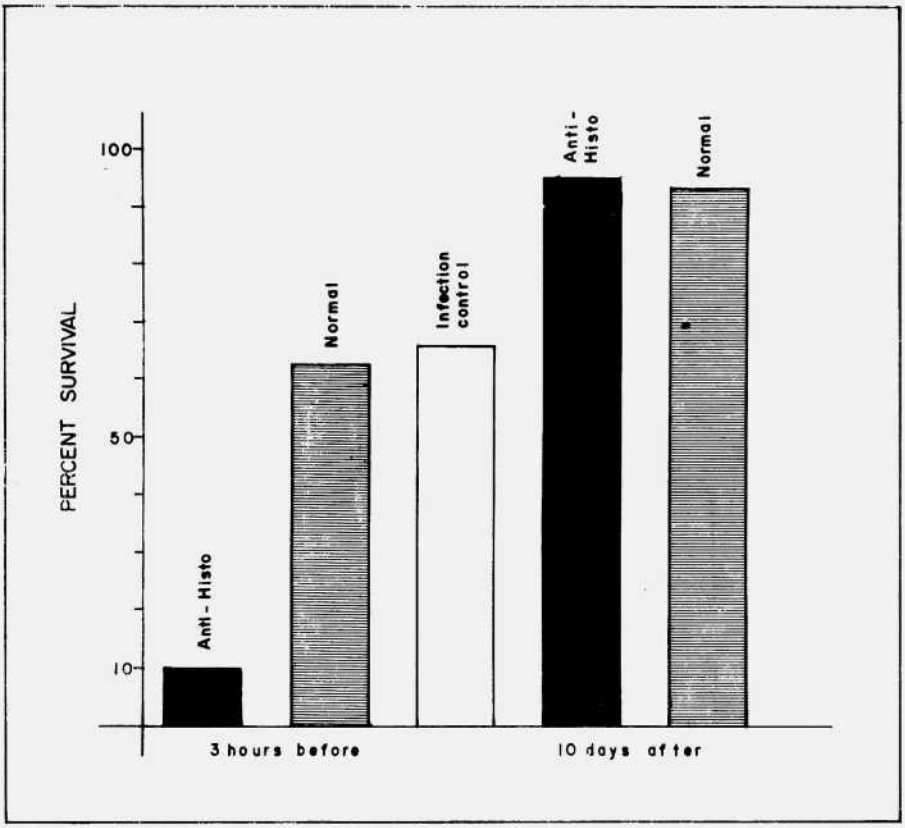

FIG. 3. PERCENT SURVIVAL OF MALE ADULT BALB/c MICE INFECTED WITH *Histoplasma capsulatum* AND TRANSFERRED PASSIVELY WITH ANTI-Histo-*plasma* SERA. Mice were infected intraperitoneally with a sublethal dose (1.9×10^8 yeast cells ml^{-1}) of a low virulent strain of *Histoplasma capsulatum* No. 5037. Passive serum was done subcutaneously with 0.3 ml of normal or anti-*Histoplasma* serum, containing high antibody titres detected by ELISA. ■ Anti-*Histoplasma* serum ▤ Normal serum; □ Control of infection with no transfer.

We know that antibodies are not the only serum factors that could interfere with the cellular response, non specific factors and non immunoglobulin factors have also been reported (Bullock and Fasal, 1971; Levene and Turk, 1969), perhaps explaining why we observed the influence of normal sera on the abrogation of DTH. However, there is an abundance of evidence which strongly support the regulatory function of antibody molecules, either by mechanisms which involve specificity or by cross reactivity.

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