

***In vitro* cell response of *Treponema pallidum*-infected rabbits**

II. INHIBITION OF LYMPHOCYTE RESPONSE TO PHYTOHAEMAGGLUTININ BY SERUM OF *T. PALLIDUM*-INFECTED RABBITS

VICTORIA WICHER & K. WICHER *Department of Microbiology, School of Medicine, SUNY, Buffalo, New York, and Division of Clinical Microbiology and Immunology, Erie County Laboratory, E. J. Meyer Memorial Hospital, Buffalo, New York, U.S.A.*

(Received 12 March 1977)

SUMMARY

Serum inhibitors of lymphocyte response to PHA were found in *T. pallidum*-infected rabbits. The humoral inhibitors could be detected as early as 10 days after infection and persisted for at least 6 months. The factors also suppressed the allogeneic lymphocyte response. Control, or normal, rabbit sera likewise contain serum inhibitors, but in much lower concentration. The detection of the humoral inhibitors depended on the susceptibility of the indicator lymphocytes. Cells of some rabbits were more sensitive to the inhibition than others. In addition to serum inhibitors, lymphocytes of *T. pallidum*-infected animals seem to be impaired and responded to PHA less vigorously than cells of normal rabbits. The inhibitory activity is most likely the result of a complex group of substances with different physicochemical characteristics; some pre-exist and others are newly formed after infection. Problems associated with the detection of such inhibitors are discussed.

INTRODUCTION

Serum inhibitors of lymphocyte response to phytohaemagglutinin (PHA) have been observed in several diseases associated with impaired cell-mediated immunity (Bullock & Fasel, 1971; Knowles *et al.*, 1968; Mangi, Dwyer & Kantor, 1974). Recent studies of cell-mediated response in syphilis have provided evidence of cellular suppression of mitogens and/or antigens in naturally (Levene *et al.*, 1969; Musher, Schell & Knox, 1974) or experimentally (Pavia, Folds & Baseman, 1976; Wicher & Wicher, 1977) infected hosts. Humoral inhibitors of lymphocyte functions have been reported in patients with primary, secondary and tertiary syphilis (Dwyer *et al.*, 1975; Levene *et al.*, 1969), although conflicting results have been observed by others (Musher *et al.*, 1974). The complexity of the immunopathological response taking place during *T. pallidum* infection and the limitation imposed by using human material might explain the discordant results. The present studies demonstrate in rabbit serum inhibitor(s) of lymphocyte function and expose problems associated with the demonstration of such inhibitor(s).

MATERIALS AND METHODS*

Animals. New Zealand rabbits, 3-4 kg each, were infected intratesticularly (i.t.) with approximately 2×10^7 *T. pallidum* (Nichols strain). Control animals were injected i.t. with 1 ml of saline extract of normal rabbit testis mixed with equal volume of *T. pallidum*-free saline extract of infected testis. For the sake of convenience, this control group will be referred to as animals injected with extract of normal rabbit testis (NRT). All animals were handled exactly as described in the preceding

* The sources and abbreviations of reagents, if not specified, have been given in the preceding publication.

Correspondence: Dr Konrad Wicher, Erie County Laboratory, Division of Clinical Microbiology and Immunology, E. J. Meyer Memorial Hospital, 462 Grider Street, Buffalo, New York 14215, U.S.A.

publication and the infected rabbits showed the same features of orchitis and serological response (Wassermann and treponemal antibodies). Control animals showed no testicular change or production of any type of antibodies.

Lymphocyte transformation. Lymphocytes separated by the method of Böyum (1968) were washed twice with HBSS, and resuspended in RPMI 1640 (Grand Island Biological, Grand Island, N.Y.) containing 50 u/ml of penicillin, 50 µg/ml of streptomycin and 20% of freshly drawn autologous or homologous serum. Cell suspensions containing 5×10^5 lymphocytes in 1 ml were cultured in triplicate in the presence of 5 µg of PHA and incubated for 3 days at 37°C in an atmosphere containing 5% CO₂. The optimal concentrations of PHA was established in a dose-response experiment using the lymphocytes of ten normal rabbits. 24 hr before harvesting, 1 µCi of ³[H]TdR was added and processed as described in the preceding publication. The counts per minute (ct/min) of triplicate samples were averaged and the results expressed as per cent of stimulation represented by:

$$\frac{\text{ct/min of cultures in homologous serum}}{\text{ct/min of cultures in autologous serum}} \times 100.$$

Response of normal rabbit lymphocytes to PHA in the presence of serum from randomly selected T. pallidum-infected rabbits. Purified peripheral lymphocytes (PPL) from five normal male rabbits were examined for their *in vitro* response to PHA in the presence of autologous and homologous sera obtained from seventy-five animals infected with *T. pallidum* and homologous sera obtained from twenty non-infected control male rabbits.

Kinetic appearance of serum inhibitory activity. PHA response of PPL from two selected normal rabbits (donor A and B) was examined in the presence of autologous serum, or homologous sera from six rabbits prior to, and 1, 2 and 3 months after, infection with *T. pallidum*, or homologous sera from six control rabbits prior to, and 1, 2 and 3 months after, i.t. injection with NRT.

Response of infected rabbit lymphocytes to PHA in the presence of normal rabbit serum. PPL from six infected rabbits and six control animals were examined for *in vitro* response to PHA in the presence of autologous sera and homologous sera of the two normal rabbits (A and B).

Effect of dialysis on the serum inhibitory activity. Aliquots of sera from infected and normal animals (A and B, the same as above) were dialysed against PBS 7-2, RPMI 1640, or HBSS at 4°C for 48 hr. Dialysed and non-dialysed samples were centrifuged at 27,000 g for 30 min and filtered through 0.45 µm Millipore filters. PPL from donors A and B were examined for the response to PHA in the presence of dialysed and non-dialysed autologous and homologous sera. Additionally, PPL of donors A and B were cultured with 15% autologous serum plus an increasing volume (0.1–0.3 ml/1 ml of culture) of HBSS-dialysed homologous serum of infected or control animals.

Effect of heat treatment on serum inhibitory activity. Sera from infected animals and healthy rabbits A and B were inactivated for 30 and 60 min at 56°C. Fresh and inactivated sera were centrifuged at 27,000 g for 30 min and filtered through a 0.45 µm Millipore filter. Lymphocytes from rabbits A and B were cultured in the presence of PHA and these samples (20% v/v).

The *in vitro* lymphocyte transformation technique was in all experiments the same except for the change of the sera in the cell cultures. Whenever and wherever examination of viability of cells was needed, the trypan blue exclusion test was done counting 300–500 cells. All results were statistically analysed by the Students' *t*-test.

Effect of serum from infected animals on allogeneic lymphocyte stimulation. A one-way mixed lymphocyte culture (MLC) was done using PPL from a normal rabbit as responder (R) and mitomycin (25 µg/10⁷ cells) treated lymphocytes from another rabbit as stimulator cells (Sm). 0.5 ml of cell suspension (0.5×10^6) from each animal were mixed and incubated for seven days in the presence of 15% fresh responder's serum. 4 hr before harvesting, the cells were centrifuged at 220 g, washed twice with HBSS, resuspended in fresh RPMI 1640 containing 20% fresh autologous or homologous sera from infected (3 months after infection) or control (3 months after injection) rabbits and the [³H]TdR added. Triplicate or quadruplicate samples were used. The DNA synthesis was examined as in the experiments described previously.

Lymphocytotoxic antibody test. The method of Terasaki, Mottironi & Barnett (1970) with slight modifications was used. Lymphocytes from donors A and B were washed twice with HBSS and resuspended to a concentration of 10⁶ cells/ml in magnesium (Mg²⁺) and calcium (Ca²⁺) containing gelatin-veronal buffer (GVB²⁺). Sera from infected, control (NRT), and ten healthy rabbits were used fresh and after 30 min inactivation at 56°C. For this test, sera were used neat and diluted 1:5. As complement, fresh normal rabbit or guinea-pig sera, previously adsorbed with normal rabbit erythrocytes, were used. The test was performed as follows: to 0.1 ml of serum, 0.1 ml of cell suspension was added and incubated for 30 min at 15°C. Complement at a dilution of 1:5, 1:10 and 1:20 was added and the mixture incubated for 3 hr at 37°C. Cytotoxicity was assessed by trypan blue exclusion counting 300–500 cells. A positive control using a specific guinea-pig anti-rabbit lymphocyte serum was included in each test.

RESULTS

Response of normal rabbit lymphocytes to PHA in the presence of serum from randomly selected T. pallidum-infected rabbits

The lymphocyte response in the presence of homologous sera of normal rabbits varied from 50–140%

(mean 95%) as shown in Fig. 1. Much wider variation, from almost complete inhibition (90%) to high stimulation (approximately 400%) was observed using sera from *T. pallidum*-infected animals. Significant inhibition or stimulation was observed in all groups, but the reduced response to PHA was more frequently seen with sera of animals infected for up to 6 months than with sera of rabbits infected for 1 or 2 years. The latter group showed, rather, an increase in lymphocyte stimulation. In further experiments, two normal rabbits (donors A and B) which, in the above-mentioned experiments, demonstrated differences in the mitogenic response when cultured with identical sera, were selected as lymphocyte donors. The cells were examined for the PHA response in culture with autologous and homologous sera from six healthy rabbits and six animals infected for 2 months. The results (Fig. 2) indicated that the

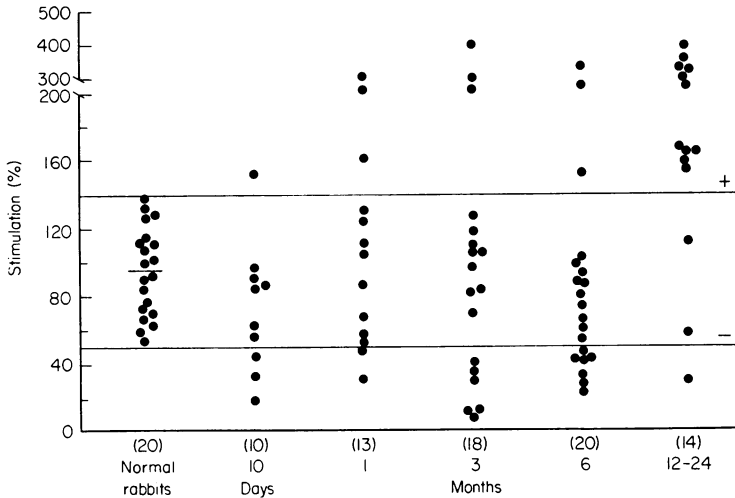


FIG. 1. Lymphocytic response to PHA. Effect of sera of randomly selected *T. pallidum*-infected rabbits on normal rabbit lymphocytes. The results are expressed as percentage stimulation. The numbers in parentheses indicate number of sera examined and those below, time of infection in days or months. The two horizontal lines (marked + and -) indicate values of ± 2 s.d. The lymphocyte response to PHA in the presence of homologous sera of normal rabbits ranged from 50 to 140% with a group mean of 95%. The response to PHA in the presence of sera from infected animals demonstrated wide variations, from significant inhibition to stimulation.

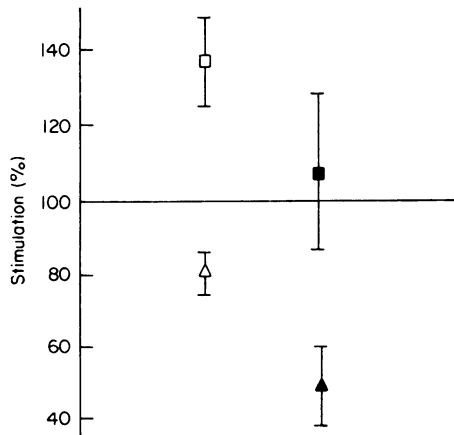


FIG. 2. Lymphocytic response to PHA. Cells from two normal rabbits, donors A (□ and ■) and B (△ and ▲) were examined for their response to PHA in the presence of homologous sera of six normal rabbits (□ or △) and six rabbits infected with *T. pallidum* (■ or ▲). The results are expressed as mean value of percentage stimulation of the group \pm s.e.m. The lymphocytes of donor B, whether cultured in the presence of normal sera or sera of infected animals, were more sensitive to inhibition than lymphocytes of donor A.

response may depend upon the donor's lymphocytes: donor B's lymphocytes, whether in the presence of normal or infected animal sera, were more inhibited than donor A's cells. Although the inhibition of PHA response was more pronounced in cultures containing sera of infected animals, significant suppression was observed only with cells of donor B ($P < 0.05$).

Kinetics of the appearance of serum inhibitory activity

The PHA response of lymphocytes of donor A (Fig. 3a) and B (Fig. 3b) in cultures containing auto-logous or homologous sera obtained at varying intervals after injection was examined. The results indicated that the response of donor A lymphocytes in the presence of sera from infected animals was not significantly inhibited by samples drawn in the first 2 months of infection, results which are similar to those obtained with sera of the control animals injected with NRT. Significantly ($P < 0.001$) reduced response to PHA was observed only with sera obtained 3 months after infection. The slightly reduced response to PHA in the presence of sera from control animals 2-3 months after injection was not significant. The lymphocytes of donor B responded differently. Good PHA stimulation was observed in the test with pre-infection sera (mean $145 \pm 18\%$) but not with the sera of the control group (mean $82 \pm 5.0\%$). After *T. pallidum* infection, the sera caused a significantly ($P < 0.005$) reduced response to PHA throughout the 3 months of examination. The sera from the control animals did not significantly affect the response of the lymphocytes.

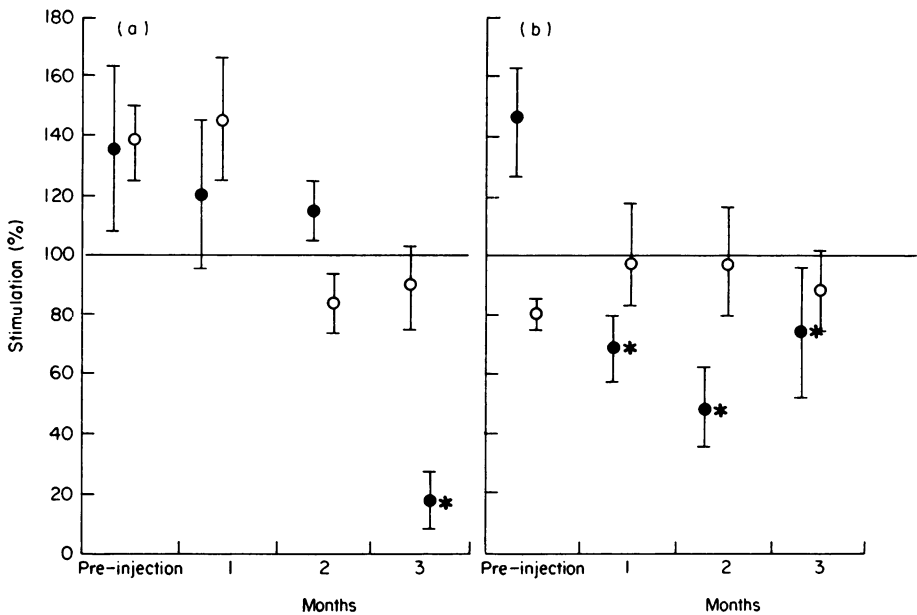


FIG. 3. Kinetics of the appearance of serum inhibitory activity. (a) Response of donor A lymphocytes to PHA in the presence of sera of six *T. pallidum*-infected rabbits (●) and sera of six control rabbits injected i.t. with saline extract of normal rabbit testes (○). Results are expressed as mean value of the group \pm s.e.m. Significantly (*), reduced response to PHA was observed only with sera from rabbits infected for 3 months. Sera of the control animals did not cause significant reduction in response. (b) Response of donor B lymphocytes to PHA. For description see Fig. 3a. Significantly reduced responsiveness to PHA was observed with sera of rabbits being infected from 1 to 3 three months. Sera of the control animals did not affect the lymphocyte response to PHA.

Response of infected rabbit lymphocytes in the presence of normal rabbit serum

The results are shown in Fig. 4a and 4b. The lymphocytes of the control animals demonstrated a normal response with acceptable variations in both homologous sera of donors A and B. Although the lymphocytes of the infected animals appeared to be much more stimulated in the presence of either serum A or B, the degree of stimulation was not statistically significant. This is probably due to the inability of lymphocytes from infected rabbits to respond normally.

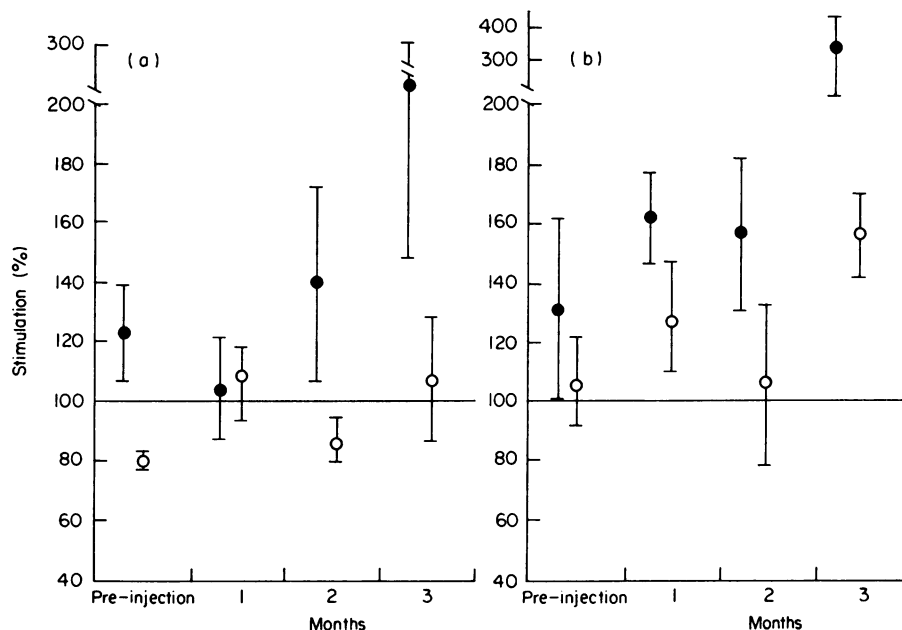


FIG. 4. Response of *T. pallidum*-infected rabbit lymphocytes. (a) Cells of six infected animals (●) and six controls injected with saline extract of normal rabbit testes (○) were examined for PHA response in the presence of autologous and homologous serum of donor A. Horizontal line indicates 100% of stimulation. Results are expressed as mean value of the groups \pm s.e.m. The cells of the control animals responded within normal variations. The lymphocytes of the infected animals appear to be more stimulated but the response was not significant (detailed explanation see text). (b) Lymphocytes of infected and control rabbits examined for PHA response in the presence of autologous and homologous serum of donor B (for description see Fig. 4a).

Effect of dialysis on serum inhibitory activity

In general terms, similar results were observed for untreated or RPMI-dialysed sera in the autologous and homologous systems (Table 1). However, cells cultured in the presence of PBS-dialysed sera showed severe impairment of PHA response albeit their good viability (46–63%).

To explore the presence of non-dialysable inhibitory substances in the sera, lymphocytes of normal rabbits were cultured in the presence of PHA, 15% fresh autologous serum and increasing volumes (0.1–0.3 ml per 1 ml of culture) of HBSS-dialysed sera of infected or control rabbits (Table 2). The cells

TABLE 1. Effect of dialysis on serum inhibitory activity

Cell donor	Inhibitory sera		Cell response in sera (ct/min)		
	Month of infection	Condition	Autologous	Homologous	Stimulation (%)*
A	3	Non-dialysed	10899† (9740–12058)	4785† (3485–5788)	44† (36–53)
		PBS-dialysed	263 (199–296)	106 (83–124)	—
		RPMI-dialysed	10241 (9350–11325)	5798 (3765–7480)	56 (40–66)
B	2	Non-dialysed	6125 (3130–9121)	2376 (873–3880)	39 (28–43)
		PBS-dialysed	154 (110–199)	124 (99–150)	—
		RPMI-dialysed	7755 (3950–11560)	3490 (780–6200)	45 (19–53)

Numbers in parentheses show the ranges of the results.

* $\frac{\text{ct/min of cells in homologous serum}}{\text{ct/min of cells in autologous serum}} \times 100$.

† Mean values.

TABLE 2. Effect of increasing volume of dialysed homologous serum on rabbit lymphocyte response to PHA

Inhibitory sera*		Cells cultured in sera		Stimulation (%)	
Group	Number	Autologous, fresh†	Homologous, dialysed‡	Mean	Ranges
<i>T. pallidum</i> -infected	3	0.15§	0.1§	51	25-72
		0.15	0.2	35	18-60
		0.15	0.3	21	10-30
Control, injected with NRT	3	0.15	0.1	85	84-86
		0.15	0.2	65	59-69
		0.15	0.3	60	56-65

Stimulation results as in Table 1.

* Sera collected at 3 months after injection.

† Autologous: serum of lymphocyte donors was present in all cultures as nutrient.

‡ Homologous: serum of infected or control animals dialysed against HBSS.

§ Volume in ml/1 ml of culture.

cultured with the dialysed sera (0.3 ml) of infected rabbits showed a stimulation ranging from 10-30% (inhibition 70-90%), whereas the stimulation of cells incubated with sera (0.3 ml) of control animals ranged from 56-65% (inhibition 35-44%). The inhibition in both groups paralleled the volume of sera added.

Effect of heating on serum inhibitory activity

The heating of serum (autologous or homologous) at 56°C for 30 min enhanced its ability to sustain lymphocyte DNA synthesis as compared with fresh serum (Table 3). In three out of eight homologous sera the inhibitory activity was lost almost completely (96-99% of stimulation). Heating also increased 3-10 times the PBS-dialysed serum supporting capability (data not presented in Table 3).

Effect of infected rabbit serum on allogeneic lymphocyte stimulation

The serum inhibitory action on DNA synthesis was examined in a one-way MLC (Table 4). The allogeneic response was partially inhibited by the homologous sera of infected rabbits (40-59%) but not by the sera of control animals (2-5%).

TABLE 3. Effect of heating on serum inhibitory activity

Cell donor*	Inhibitory sera		Stimulation (%)	
	Number	Condition	Mean	Ranges
A	4	Fresh	49	36-56
		Inactivated	75	40-99
B	4	Fresh	40	35-44
		Inactivated	61	28-96

Stimulation results as in Table 1.

* Lymphocytes were cultured in autologous sera and homologous sera (inhibitory) from *T. pallidum*-infected rabbits collected 2 and 3 months after infection.

Lymphocytotoxic antibodies

Sera obtained from infected, control and healthy rabbits were examined for lymphocytotoxic antibodies. The sera from the infected or control animals were taken at various times after injection. Regardless of whether rabbit or guinea-pig complement was used, the lymphocytes employed as target cells were not affected by the sera tested. The number of dead cells varied between 4–8% as compared to the 98–100% of dead cells in the positive control using guinea-pig anti-rabbit lymphocyte serum.

TABLE 4. Effect of serum inhibitory activity on allogeneic lymphocyte stimulation*

Inhibitory sera		Stimulation (%)	
Group	Number	Mean	Range
<i>T. pallidum</i> -infected	3	50	41–60
Control, injected with NRT	3	100	95–108

Stimulation results as in Table 1.

* Responder (R) and stimulator (Sm) lymphocytes were cultured for 7 days in the R's serum. Homologous sera of infected or control rabbits were added 4 hr prior to harvest along with [³H]Tdr.

DISCUSSION

The present study showed that *T. pallidum* infection in the rabbit induces an increase in the production of serum inhibitors of lymphocyte response to PHA. Sera of control animals (injected with NRT) showed non-significant levels of inhibitors as did the sera of normal rabbits. Our results confirmed the findings of Levene *et al.* (1969) and Dwyer *et al.* (1975) who observed inhibition of DNA synthesis in PHA-stimulated lymphocytes in secondary syphilis in man. Difficulties encountered by other investigators (Musher *et al.*, 1974) in demonstrating such activity may be due to the degree of infection as well as to the difficulty of controlling variations in the cell response to the mitogen, even when using normal human serum (Mangi *et al.*, 1974; Bredt & Mardiney, 1969).

Inhibitory and stimulatory substances may co-exist in sera of normal subjects (Waksman & Namba, 1976; Bredt & Mardiney, 1969) and, therefore, the degree of lymphocyte response will depend upon a series of competitive and regulatory interactions. This was also apparent in our experimental model. When lymphocytes of five randomly selected normal rabbits were cultured with an optimal concentration of PHA in the presence of autologous or homologous sera from seventy-five *T. pallidum*-infected animals (Fig. 1), wide variations ranging from significant inhibition to significant stimulation were observed. In addition, a number of sera could reduce or enhance the response to the mitogen depending upon the donor lymphocytes. This was clearly demonstrated by the kinetic studies (Fig. 3). Detection of the early minimal inhibition required the use of sensitive indicator lymphocytes, a property varying from donor to donor (Fig. 2). The preliminary experiments (Fig. 1) demonstrated that humoral inhibitors are already present 10 days after infection and persist for at least 6 months. The depressed responsiveness to PHA is also influenced by an impairment of the lymphocyte function. Cells of infected animals responded to PHA better in normal serum-containing cultures but not to the expected degree. These results are in agreement with our previous findings (Wicher & Wicher, 1977) of suppressed mitogenic response of lymphocytes cultured in the presence of autologous serum obtained before infection.

A plethora of humoral and cellular factors with immunoregulatory properties are known. Some of them are normally present in the serum, i.e. immunoregulatory alpha-globulin (IRA); others arise as a consequence of inflammation, i.e. C-reactive protein (see review by Waksman & Namba, 1976). In our

experiments, the suppressor substances were effective in reducing the DNA synthesis of PHA-stimulated and also allogeneically stimulated lymphocytes, implying that the inhibitory activity is not just due to competition with mitogen (Morse, 1968).

The biological rôle of these inhibitors is unknown, although they correlate with progressive infection and suppressed cellular response (Pavia *et al.*, 1976; Wicher & Wicher, 1977). These inhibitors may be related to the load of treponemal antigen in circulation or to unidentified blocking-type antibody (Levene *et al.*, 1969). Our attempts to detect the presence of lymphocytotoxic antibodies were unsuccessful.

A marked increase in DNA synthesis was observed when lymphocytes were cultured in the presence of heat-inactivated sera (56°C for 30–60 min). This was observed with sera of infected and normal animals indicating that thermolabile inhibitory substances might be present in the physiological, as well as the pathological, conditions. Thermolabile immunoregulatory substances have been reported in human serum (Ford, Caspary & Shenton, 1973; Forsdyke, 1973), although the increase in the stimulatory activity in our experiments exceeded (3–12-fold increase) that found in man (Yachnin & Raymond, 1975). Individual variations in the responsiveness to heat treatment were observed in our experiments, Sera of three out of eight animals, after a 30 min heating, almost completely lost the inhibitory properties, whereas the remaining five sera, after heating, demonstrated higher stimulatory properties when compared with the fresh sera. However, the increase was proportional in both the autologous and homologous sera, and therefore the percentage stimulation remained the same. These results led us to assume that not all inhibitory substances are thermolabile, or a deficiency in stimulatory factors is responsible, or both.

The serum inhibitory properties were further examined by dialysing the sera against different solutions. The use of PBS-dialysed sera from normal, control or infected animals caused a substantial lowering in the nutritional properties of the lymphocytes. A similar phenomenon observed in a human system has been attributed to the loss of low-molecular-weight nutritional substances, particularly Ca^{2+} (Alford, 1970). This was corroborated by us when using sera dialysed against RPMI. The PHA-lymphocyte stimulation in autologous, and especially in homologous, sera was good and, in several instances, even better than in native sera. When increasing volumes of HBSS-dialysed serum from infected and control animals were added to lymphocytes cultured in fresh normal rabbit serum, a definite rise in the inhibition was observed (Table 2), being more pronounced with the infected (70–90%) than the control sera (35–44%). This would indicate that non-dialysable macromolecular immunosuppressive substances are retained, although it is possible that some low-molecular-weight substances are lost during dialysis as suggested from the experiments with RPMI-dialysed sera, which demonstrated a slight increase in DNA synthesis.

Unsaturated fatty acids (Field & Shenton, 1975) and prostaglandins (Okazaki *et al.*, 1976) are among other small molecular substances that have been shown to be very effective in depressing lymphocyte function. These findings should be kept in mind in *T. pallidum* infection where obviously the lipid metabolism is activated in the early stage (Kumar & Wicher, 1975), and most probably altered to the extent of triggering the host response to a very common cellular component, cardiolipin. We might speculate, therefore, that the serum inhibitory activity observed in *T. pallidum*-infected rabbits is due to a complex set of substances with different physicochemical characteristics. Some of these exist under normal conditions but increase after treponemal infection. They may be products of the invasive organisms and/or products of the host cells released in response to the infection.

We thank Judy Martinko for her excellent technical assistance. This work was supported by grant R01-AI-11102 from the National Institute of Health, United States Public Health Service.

REFERENCES

- ALFORD, R.H. (1970) Metal cation requirements for phyto-hemagglutinin-induced transformation of human peripheral lymphocytes. *J. Immunol.* **104**, 698.
- BÖYUM, A. (1968) Separation of leukocytes from blood and bone marrow. *Scand. J. clin. Lab. Invest.* **21**, Suppl. 97, 7.

- BREDT, A.B. & MARDINEY, M.R., JR (1969) Reactivity of human lymphocytes to autologous and homologous serum. *J. Immunol.* **102**, 1526.
- BULLOCK, W.E., JR & FASAL, P. (1971) Studies of immune mechanism in leprosy. II. The rôle of cellular and humoral factors in impairment of the *in vitro* immune response. *J. Immunol.* **106**, 888.
- DWYER, J.M., THOMPSON, J., MANGI, R., & LEE, R. (1975) Suppression of the lymphocyte response to PHA by serum from subjects with syphilis. *Fed. Proc.* **34**, Abstract, 996.
- FIELD, E.J. & SHENTON, B.K. (1975) Inhibitory effect of unsaturated fatty acids on lymphocyte-antigen interaction with special reference to multiple sclerosis. *Acta Neurol. scand.* **52**, 121.
- FORD, W.H., CASPARY, E.A. & SHENTON, B. (1973) Purification and properties of a lymphocyte-inhibition factor from human serum. *Clin. exp. Immunol.* **15**, 169.
- FORSDYKE, D.R. (1973) Serum factors affecting the incorporation of [³H]thymidine by lymphocytes stimulated by antigen. III. Evidence for a rôle of complement from studies with specific complement inhibitors. *Immunology*, **25**, 613.
- KNOWLES, M., HUGHES, D., CASPARY, E.A. & FIELD, E.J. (1968) Lymphocyte transformation in multiple sclerosis. *Lancet*, *ii*, 1207.
- KUMAR, V. & WICHER, K. (1975) Changes in the lipids in organs of rabbits infected with *Treponema pallidum*. *Abstracts of the annual meeting of the American Society for Microbiology*, **73**, E69.
- LEVENE, G.M., TURK, J.L., WRIGHT, D.J. & GRIMBLE, A.C.S. (1969) Reduced lymphocyte transformation due to plasma factor in patients with active syphilis. *Lancet*, *ii*, 246.
- MANGI, R.J., DWYER, J.M. & KANTOR, F.S. (1974) The effect of plasma upon lymphocyte response *in vitro*. Demonstration of a humoral inhibitor in patients with sarcoidosis. *Clin. exp. Immunol.* **19**, 519.
- MORSE, J.H. (1968) Immunological studies of phytohemagglutinin. I. Reaction between phytohemagglutinin and normal sera. *Immunology*, **14**, 713.
- MUSHER, D.M., SCHELL, R.F. & KNOX, J.M. (1974) *In vitro* lymphocyte response to *Treponema refringens* in human syphilis. *Infect. Immunity*, **9**, 654.
- OKAZAKI, T., WICHER, K., SHIMIZU, M. & ARBESMAN, C.E. (1976) The rôle of prostaglandin E in response of human lymphocyte sub-population to PHA. *Fed. Proc.* **52**, Abstract, 2129.
- PAVIA, C.S., FOLDS, J.D. & BASEMAN, J.B. (1976) Depression of lymphocyte response to concanavalin A in rabbits infected with *T. pallidum* (Nichols strain). *Infect. Immunity*, **14**, 320.
- TERASAKI, P.I., MOTTIRONI, V.D. & BARNETT, E.V. (1970) Cytotoxins in disease: autocytoxicity in lupus. *New Engl. J. Med.* **283**, 724.
- WAKSMAN, B.H. & NAMBA, Y. (1976) On soluble mediators of immunologic regulation. *Cell. Immunol.* **21**, 161.
- WICHER, V. & WICHER, K. (1977) *In vitro* cell response of *Treponema pallidum*-infected rabbits. I. Lymphocyte transformation. *Clin. exp. Immunol.* **29**, 480.
- YACHNIN, S. & RAYMOND, J. (1975) An absolute requirement for the serum macromolecules in phytohaemagglutinin-induced human lymphocyte DNA synthesis. *Clin. exp. Immunol.* **22**, 153.