INFECTION AND IMMUNITY, Aug. 1980, p. 575-582
0019-9567/80/08-0575/08$02.00/0

Vol. 29, No. 2

Detection of Circulating Immune Complexes in the Sera of Rabbits with Experimental Syphilis: Possible Role in Immunoregulation

ROBERT E. BAUGHN,[1,2]* KENNETH S. K. TUNG,[3] AND DANIEL M. MUSHER[1,3]

Departments of Microbiology and Immunology, Dermatology, and Medicine, Baylor College of Medicine, Houston Texas 77025[1]; the Infectious Disease Section (Syphilis Research Laboratory), Houston Veterans Administration Medical Center, Houston, Texas 77211[2]; and the Department of Pathology, University of New Mexico College of Medicine, Albuquerque, New Mexico 87131[3]

The in vivo and in vitro immunoglobulin G plaque-forming cell responses to sheep erythrocytes (SRBC) are nearly obliterated during disseminated syphilitic infection (3 to 8 weeks post-intravenous injection) in rabbits. Splenic and lymph node cells obtained from infected rabbits during this time period were capable of suppressing the normal in vitro responses of uninfected, SRBC-primed cells. Cell-free washings of cells from infected animals were also suppressive. This finding coupled with the fact that treatment of infected cells with proteolytic enzymes abrogated the suppressive effect constitute arguments against involvement of a specific suppressor cell population. The incidence of elevated levels of circulating immune complexes in the sera of rabbits with disseminated disease was also significantly different from that of uninfected controls or infected rabbits before the onset or after the regression of lesions. When added to cultures of lymphocytes from uninfected, SRBC-sensitized rabbits, sera containing complexes caused dose-related suppression of the in vitro immunoglobulin responses. Unlike immune complexes, no correlation was found between the presence of mucopolysaccharide materials and the stage of infection or the ability of serum to suppress the immunoglobulin responses to SRBC.

Despite years of intensive research on the immune response to infection with *Treponema pallidum,* the failure of host defense mechanisms to eradicate the infecting organism remains unexplained. The progression of syphilis from localized (primary) to disseminated (secondary) infection, the persistence of disseminated disease for weeks to months, and the occurrence of relapses of secondary infection in up to 20% of untreated patients (6) all point to an inability of the immune system to recognize and/or respond appropriately to this virulent organism. Although abundant antibodies, both treponemal and nontreponemal, appear in syphilis, an appropriate and specific antibody which might eradicate *T. pallidum* is not produced, at least not in the early stages of infection. Our laboratory has investigated the possibility that suppression of immune responsiveness might explain the unusual evolution of syphilitic infection. We have shown that in vivo immunoglobulin G (IgG) response to sheep erythrocytes (SRBC), a T-dependent antigen, is virtually obliterated in syphilitic rabbits (2). IgM responses are normal or, in fact, somewhat increased. Subsequent studies using SRBC in vitro to sensitize lymphocytes from syphilitic rabbits showed suppression of both IgG and IgM responses (3).

Several different mechanisms might contribute to the observed immunosuppression. Abnormalities in T-cell function have been suggested by earlier studies showing abnormal lymphocyte responses in vitro (15, 16, 18, 27). In certain studies, factor(s) present in the plasma or serum of patients with secondary syphilis (10, 11) or experimentally infected animals (17-19, 26-28) has been thought to play a role. Our studies (2, 3), which showed that suppression of the IgG response to SRBC was greatest at the time of maximum antigenic burden in the tissues, led us to postulate that circulating immune complexes (CIC) might be responsible. The attractiveness of this hypothesis was further enhanced by two recent findings: (i) the demonstration of elevated levels of CIC in patients with secondary syphilis (23), and (ii) the fact that bound complexes on the surface of T lymphocytes suppress the differentiation and proliferation of B cells in the presence of mitogen (14). More recently, mucopolysaccharide (MPS) material present both in serum and in the testicular fluid of infected rabbits has been shown to inhibit the mitogenic

response of normal rabbit lymphocytes to concanavalin A (4), and the contribution of this substance in immunoregulation also must be clarified.

The present paper provides data which cast additional light on these hypotheses. Evidence is presented which suggests a possible role for CIC in the observed immunosuppression. Lymphocytes from syphilitic rabbits are shown to suppress immunoglobulin plaque-forming cell (PFC) responses of normal lymphocytes in vitro. Pronase or trypsin treatment of these cells elutes the immunosuppressive factor(s), whereas hyaluronidase does not. CIC are found to be present in the serum of syphilitic rabbits, and a strong association exists between the intensity of disseminated lesions, levels of CIC, and suppression of the in vitro PFC response. Although MPS materials are also detected in the serum of infected rabbits, they do not appear to play a direct role in the observed immune suppression.

MATERIALS AND METHODS

Infection and sensitization of rabbits. In most experiments, outbred New Zealand White male rabbits or inbred strain III VO/J animals (Jackson Laboratories, Bar Harbor, Me.) were infected intravenously (i.v.) with 4×10^7 *T. pallidum* as described previously (2, 3). In certain studies, however, animals were infected intradermally with 10^7 per site or intratesticularly with 10^7 organisms per testes. Uninfected control rabbits received the same number of heat-killed *T. pallidum* (60°C for 2 h). At appropriate time intervals rabbits were sensitized with one or two i.v. injections of 2×10^9 SRBC. Serum samples, obtained at various stages of infection, were separated from clotted blood after 2 h at room temperature and stored in aliquots at −70°C.

Collection, separation, treatment, and culturing of lymphocytes. Single-cell suspensions of splenic, peripheral blood, or lymph node lymphocytes were obtained as previously described (2, 3). Monocyte depletion was achieved by passage through Sephadex G-10 (Pharmacia Fine Chemicals, Inc., Piscataway, N.J.) columns using a modification of the method of Ly and Mishell (12). Collection of adherent cell populations for culture was made by allowing spleen cells to adhere to glass petri dishes for three 30-min incubation periods and removing attached cells with the aid of a rubber policeman. Enzymatic treatment of lymphocytes was carried out by incubating 10^7 spleen or lymph node cells in 1 ml of RPMI containing 0.1% Pronase (Calbiochem, San Diego, Calif.), 0.25% trypsin (GIBCO Laboratories, Grand Island, N.Y.), or 0.1 mg of ovine hyaluronidase (Sigma Chemical Co., St. Louis, Mo.) for 60 min at 37°C with gentle rocking. Cell suspensions were then diluted 1:2 with cold RPMI and centrifuged at $200 \times g$ for 10 min. Cell pellets were then washed four times in cold RPMI before cell counting. In those studies using inbred animals, cells from infected rabbits were cocultured with normal cells, whereas in the other experiments cells were obtained from normal, VDRL nonreactive rabbits 7 days after SRBC sensitization. The only other modifications in the conditions for cell culture (3) were that increasing microliter concentrations of sera, testicular extracts, or lymphocyte washing were added to replica cultures. Dose response curves were constructed to determine the effect of each substance on the response of primed cells in the Jerne plaque technique.

Hemolytic plaque assay. Cultured lymphocytes secreting antibody specific for SRBC were enumerated by using the Jerne plaque assay (9), as previously modified in our laboratory (2, 3). Both direct and indirect in vitro assays were performed at several dilutions on replicate pools of duplicate cultures with and without the various additions. The final results in each instance were determined by averaging four to eight separate values, and the degree of suppression was calculated on the basis of maximal responses in control cultures to which only antigen (50 μl of a 2% suspension of SRBC) had been added.

MPS. The capillary turbidity microassay method (8) was used to determine the MPS content of all test substances added to lymphocyte cultures. Attempts to depolymerize MPS in test samples were carried out by diluting the samples 1:2 in 0.02 μg of ovine hyaluronidase per ml (Sigma Chemical Co.) followed by incubation at 37°C for 30 min, followed by an additional 24 h of incubation at 4°C.

Detection of CIC. CIC in sera were assayed both by the Raji cell radioassay and the Clq solid phase assay (Clq-SPA). The Raji assay, originally described by Theofilopoulos et al. (24, 25), was modified for use with rabbit sera. Ten normal sera were included in each assay, and the mean and standard deviation of this group were determined. Test sera results were expressed as the number of standard deviations from the normal mean, and sera were considered positive when the uptake of ^{125}I-labeled goat-anti-rabbit IgG was more than 2 standard deviations above normal.

Clq was isolated from fresh normal rabbit serum by the methods of Yonemasu and Stroud (29), and the Clq-SPA was performed by a modification of the method of Hay et al. (7) using ^{125}I-labeled protein A. Protein A (Pharmacia; 100 μg in 1 ml of phosphate-buffered saline at pH 7.2) was labeled with 3 mCi of ^{125}I by adding 60 μg of chloramine T. After 20 min of incubation at 4°C, the reaction was stopped by adding sodium metabisulfite. Free iodine was removed on Sephadex G-25 (Pharmacia) columns. Labeled protein was divided into portions and stored at −70°C. The assay was performed as previously described (1). Results were expressed as for the Raji cell radioassay. Levels of sensitivity for detection of immune complexes by the Raji and Clq assay were 20 μg and 5 μg each per ml of serum, respectively.

Statistical analysis. Statistical evaluation was determined by chi-square analysis, the Mann-Whitney U Test, and Spearman's coefficient of rank correlation, r_s (22).

RESULTS

In vivo response of syphilitic, inbred rabbits to SRBC. The purpose of the initial experiments with inbred III VO/J rabbits was to show

that these animals had immunological reactivity similar to that observed in outbred rabbits (2, 3). Four animals were infected with 4×10^7 *T. pallidum*, and four sex- and age-matched controls received heat-killed *T. pallidum* i.v. Two rabbits from each group then received either one injection of SRBC 28 days later or two injections, one 21 and one 28 days later. The in vivo PFC responses of spleen cells from each of these rabbits on day 35 closely resembled those observed previously with splenic lymphocytes from outbred rabbits (data not shown); IgG responses of the infected animals were less than one-tenth of control responses, whereas the IgM responses were normal or slightly increased.

In vitro responses of cultured cells from syphilitic, inbred rabbits. Splenic or lymph node lymphocytes from the syphilitic rabbits were admixed in varying proportions with splenic lymphocytes from their uninfected SRBC-sensitized siblings and cultured in vitro, in the presence of SRBC, for either 5 or 6 days. Results from experiments in which rabbits were sensitized with SRBC once (28 days after infection) or twice (21 and 28 days after infection) are shown in Table 1. As observed previously using lymphocytes from outbred rabbits (3), cells from normal, uninfected rabbits yielded vigorous IgM- and IgG-PFC responses, whereas the PFC responses of lymphocytes from infected rabbits in comparison were substantially lower. Addition of lymph node or splenic lymphocytes from infected rabbits, even at low ratios (1 infected lymphocyte per 40 normal splenic lymphocytes), suppressed PFC responses; the most profound effects were noted in terms of the IgG-secreting cell responses. The results obtained in both experiments in which cultures were incubated for 6 days (data not shown) were similar to those shown in Table 1.

Experiments were then performed in an attempt to determine if suppression could be ascribed to either an adherent or nonadherent cell population. Normalized data from two experiments are presented in Table 2. Suppression of normal IgM- and IgG-responses was observed when adherent or nonadherent fractions of splenic cells from syphilitic rabbits were added at a ratio of 1:20; the levels of suppression were similar to those obtained with unfractionated spleen cells from infected animals. The addition of normal cells (fractionated or unseparated) to cultures of infected cells failed to enhance the abnormally low IgG-PFC responses seen when the latter were cultured alone. Depletion of adherent cells left a nonadherent population of normal cells which gave a minimal PFC response; normal responsiveness was, to a great extent, restored by adding back adherent cells at a 1:20 ratio. Similar results were obtained using cells from lymph nodes of infected rabbits (data not shown).

Enzymatic treatment of lymphocytes from syphilitic rabbits. The failure to identify a specific suppressor cell type in the adherent or nonadherent spleen and lymph node preparations of infected cells led us to hypothesize that substances bound to the membranes of adherent and nonadherent cells might be responsible for the observed immunosuppression. To test this

TABLE 1. *Suppression of the secondary in vitro response of normal rabbit splenic lymphocytes cocultured with splenic or lymph node lymphocytes from syphilitic rabbits*[a]

Cells ($\times 10^6$) added per culture			Expt 1				Expt 2			
Normal spleen cells	Infected spleen cells	Infected lymph node cells	PFC/culture		Suppression (%)		PFC/culture		Suppression (%)	
			IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
2	0	0	37,333	21,333			28,333	46,333		
0	2	0	4,627	186			8,400	400		
0	0	2	106	33			260	0		
1	1	0	6,600	320	82	98	12,400	1,600	56	96
1.5	0.5	0	9,467	399	75	98	15,600	2,400	45	95
2	0.1	0	11,333	2,267	70	89	17,200	7,600	39	84
2	0.05	0	14,267	2,933	62	86	19,600	11,200	31	76
1	0	1	1,667	0	96	100	9,600	800	66	98
1.5	0	0.5	9,067	0	76	100	8,800	2,000	69	96
2	0	0.1	12,933	1,067	65	95	11,600	4,400	59	91
2	0	0.05	18,667	1,599	50	93	16,400	9,200	42	80

[a] The infected rabbit in experiment 1 received 2×10^9 SRBC on day 28 of infection, whereas the infected rabbit in experiment 2 received two injections of SRBC on days 21 and 28. Control animals, which had received heat-killed *T. pallidum*, were sensitized at the same times. Seven days after sensitization, cells were placed in culture together with SRBC. Data are reported as PFC per culture on day 5.

hypothesis, lymph node and spleen cells from infected, inbred rabbits were treated with Pronase, trypsin, or hyaluronidase, extensively washed, and then admixed with splenic cells from normal SRBC-sensitized animals. In addition, the first wash fluid obtained during preparation of the lymph node and spleen cells was decanted, filtered through a series of membrane filters, and added in microliter concentrations to cultures of normal cells. The normalized results of two studies in which the infected and control inbred rabbits had received one injection of SRBC are presented in Table 3. Pronase or trypsin treatment of infected lymph node cells abolished their ability to suppress immunoglobulin responses of normal lymphocytes, whereas treatment with hyaluronidase did not alter the effect of syphilitic lymphocytes on normal cells.

TABLE 2. *Suppression of the secondary in vitro response of normal rabbit splenic lymphocytes cocultured with adherent and nonadherent splenic lymphocytes from syphilitic rabbits*[a]

Cells ($\times 10^6$) added per culture		PFC/culture	
Normal	Infected	IgM	IgG
2 (unseparated)	0	19,767	39,417
2 (unseparated)	0.1 (unseparated)	7,550	3,500
2 (unseparated)	0.1 (adherent)	5,650	5,950
2 (unseparated)	0.1 (nonadherent)	8,825	6,750
0	2 (unseparated)	11,200	1,550
0.1 (unseparated)	2 (unseparated)	14,350	1,860
0.1 (adherent)	2 (unseparated)	13,450	2,140
0.1 (nonadherent)	2 (unseparated)	10,700	1,250
2 (nonadherent)	0	2,125	440
2 (nonadherent) + 0.1 (adherent)	0	11,230	18,540
2 (nonadherent)	0.1 (adherent)	1,675	525
2 (nonadherent)	0.1 (nonadherent)	1,425	325

[a] Normalized data from two experiments with inbred rabbits are expressed as PFC per culture on day 5. In both experiments, SRBC were administered i.v. 21 and 28 days after infection with *T. pallidum*. Control animals in each experiment were sensitized 21 and 28 days after having received heat-killed *T. pallidum*.

Both proteolytic enzymes, but not hyaluronidase, exerted similar effects on spleen cells from infected rabbits (data not shown). Enzyme treatment of infected cells, however, did not enhance the ability of these cells to respond alone in culture to SRBC. Treatment of normal lymphocytes with each of the enzymes before placing the cells in culture had no effect on their ability to respond (data not shown). Of equal importance, the cell-free wash fluids of infected cells were capable of suppressing both the IgM and IgG responses of normal spleen cells (Table 4).

CIC and MPS studies. CIC were detected in 43 of 50 (86%) sera obtained between the third and seventh week after i.v. *T. pallidum* infection by the Raji cell assay (Table 5), compared with 1 of 18 (6%) sera obtained before the onset or after the regression of lesions ($P < 0.0005$, chi-square analysis); 2 of 12 (16%) preinfection sera were positive. When these same sera were as-

TABLE 4. *Effect of cell-free washings of splenic or lymph node lymphocytes on PFC responses of normal lymphocytes*[a]

Additions per culture	PFC/culture		Suppression (%)	
	IgM	IgG	IgM	IgG
None	22,367	14,700		
Normal spleen cell wash:				
50 μl	20,850	13,450	7	9
100 μl	19,900	14,100	11	4
Infected spleen cell wash:				
50 μl	10,234	2,450	54	83
100 μl	8,775	2,267	61	88
Infected lymph node cell wash:				
50 μl	16,467	3,550	26	76
100 μl	12,334	2,267	45	85

[a] Normalized data from two experiments with inbred rabbits are expressed as PFC per culture on day 5. In both experiments the normal lymphocytes were obtained from control animals sensitized with SRBC 21 days after having received heat-killed *T. pallidum*.

TABLE 3. *Pronase and trypsin abrogation of the suppressive effect of lymph node cells on the PFC response of normal rabbit splenic lymphocytes*[a]

Cells ($\times 10^6$) added per culture		Infected lymph node cells							
		Untreated		Pronase-treated		Trypsin-treated		Hyaluronidase-treated	
Normal	Infected	IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
0	2	140	30	190	30	170	30	120	40
1	1	940	0	21,400	16,500	20,600	13,600	1,080	60
1.5	0.5	1,630	80	22,200	13,800	21,700	15,000	1,260	140
2	0.1	1,810	250	21,400	14,800	20,200	15,900	2,280	300
2	0.05	3,040	290	22,800	13,700	23,200	14,200	2,620	360
2	0.01	4,250	390	23,100	14,200	22,000	18,400	4,810	460

[a] Normalized data from two experiments with inbred rabbits are expressed as PFC per culture on day 5. In both experiments, SRBC were administered i.v. 21 days after infection with *T. pallidum*. Control animals in each experiment were sensitized 21 days after having received heat-killed *T. pallidum*. PFC response of 2×10^6 normal rabbit spleen cells: IgM, 22, 367; IgG, 14,700.

sayed using the Clq-SPA, 15 of 50 (30%) samples collected between the third and seventh week were positive for CIC. In comparison, 1 of 18 (6%) sera collected before the onset of the disseminated rash or after regression of lesions were positive ($P < 0.005$, chi-square analysis), and none of the 12 preinfection sera were positive. MPS materials were demonstrated in the sera throughout the course of infection; however, there was no direct association between the levels observed and the presence of overt disseminated disease, as MPS was detectable both before the onset of lesions and after their regression.

Effect of serum containing CIC or MPS, or both, on immune responses in vitro. Rabbits were sensitized once or twice (Table 6) with SRBC. One week later, their lymphocytes were

TABLE 5. *CIC and MPS in the sera of rabbits infected i.v. with T. pallidum*

No. of rabbits	Infection status	Clinical findings	CIC[a]		MPS[b]
			Raji	Clq-SPA	
12	Preinfection	None	2/12	0/12	0/12
	Weeks postinfection:				
6	1	None	0/6	0/6	6/6 (1.0–2.0)
7	2	None	1/7 (2.36)	0/7	7/7 (0.25–2.0)
11	3	Maculo-papular rash	4/11 (2.78–5.37)	0/11	11/11 (0.5–1.0)
18	4	Widely disseminated crop of lesions (>30 in number with a diameter >12 mm); orchitis	18/18 (3.07–10.25)	5/18 (3.42–6.58)	18/18 (0.5–2.0)
10	5	Widely disseminated crop of lesions, many have coalesced; orchitis had passed	10/10 (6.93–9.37)	6/10 (3.48–9.69)	10/10 (0.5–1.0)
11	7	Widely disseminated crop of necrotic lesions, most are in various stages of healing	11/11 (5.37–8.87)	4/11 (2.11–7.50)	11/11 (0.5–1.0)
5	10	Over 90% of lesions had nearly healed, and regression in size had been noted for at least 2 weeks	0/5	1/5 (2.28)	5/5 (0.13–1.0)

[a] CIC results are expressed as the number of sera positive for CIC by the indicated assay per total number examined. Data in parentheses are expressed as the number of standard deviations above the mean for serum from normal rabbits, and the range of abnormal results is given.

[b] MPS results are expressed as the number of sera positive per total number examined. The numbers in parentheses indicate the range of positive values in milligrams per milliliter.

TABLE 6. *Suppression of the secondary in vitro response to SRBC by CIC in the serum of syphilitic rabbits*

Source of serum added to cultures	CIC[a]		MPS[b]	Amt added (μl)	Expt 1				Expt 2			
	Raji	Clq-SPA			PFC/culture[c]		Suppression (%)		PFC/culture[c]		Suppression (%)	
					IgM	IgG	IgM	IgG	IgM	IgG	IgM	IgG
None					40,500	23,100			18,400	53,600		
Preinfection	<2	<2		15	41,700	21,350	0	8	17,500	51,400	5	4
				30	39,200	22,500	3	3	16,800	52,100	9	3
				45	43,800	19,200	0	17	18,100	54,500	2	0
Infected:												
Day 7	<2	<2	1.0	15	42,100	22,800	0	1	16,900	51,200	8	4
				30	36,400	19,900	10	14	16,100	49,700	13	7
				45	37,250	20,150	8	13	17,800	50,400	3	6
Day 14	<2	<2	0.5	15	38,700	24,100	4	0	16,500	48,700	10	9
				30	41,200	21,500	0	7	17,300	52,400	6	2
				45	35,640	19,600	12	15	16,300	51,600	11	4
Day 28	10.25	3.42	1.0	15	8,900	950	78	96	12,200	8,900	34	83
				30	2,750	300	93	99	9,100	2,200	51	96
				45	1,050	50	97	>99	3,900	500	79	99
Day 35	8.87	3.48	0.5	15	12,600	1,800	69	92	10,500	10,100	43	81
				30	4,350	600	89	97	7,800	4,300	58	92
				45	1,200	110	97	99	5,100	1,100	72	98

[a] CIC data are expressed as the number of standard deviations above the mean of normal sera values.

[b] MPS results are expressed in milligrams per milliliter of serum.

[c] PFC data are expressed per culture on day 5; spleen cells from normal rabbits were obtained 7 days after a single injection of SRBC for experiment 1 and 7 days after a second injection of SRBC for experiment 2.

placed in tissue culture with increasing concentrations of sera that had been studied for quantification of CIC and MPS. Sera obtained from syphilitic rabbits 7 or 14 days after *T. pallidum* infection contained relatively high levels of MPS but did not inhibit PFC responses. By contrast, serum obtained 28 or 35 days into infection which contained the same concentration of MPS but also had high levels of CIC caused a striking depression of PFC responses with the effect being more profound in the case of IgG. This effect was dose related within the range studied. Six subsequent experiments have yielded similar data; the overall correlation between the presence of CIC and the degree of immune suppression was striking ($r^2 > 0.8$), whereas no association was seen with the presence of MPS.

DISCUSSION

Disseminated syphilis, both in the human and experimental host, is an unusual infection. The causative organism appears to fall into the niche of parasites which engenders concomitant immunity (5) in that infection, once established, persists for the life of the host despite demonstrable resistance to rechallenge. Whereas a number of mechanisms are recognized by which infectious organisms survive host defense mechanisms (5), the mechanism by which *T. pallidum* escapes remains obscure. Previous studies in our laboratory have shown that IgG responses to SRBC are depressed in syphilis (2, 3). Results of the present study extend these observations and provide evidence which suggests that CIC may be a central feature in the observed immunosuppression.

Initially, experiments were carried out to show that the immunological responses of inbred III VO/J rabbits were similar to those of outbred New Zealand rabbits which had been used previously. Sensitization of normal rabbits with SRBC induced a distinct immune response; increased numbers of IgG-secreting cells were characteristic of both the in vivo and in vitro responses. This IgG response was greatly suppressed during active syphilitic infection. An important new finding in the present study, made possible by the use of inbred rabbits, was that both lymph node and splenic lymphocytes from infected rabbits were capable of suppressing the responses of normal, SRBC-primed cells in vitro (Table 1). Initially these results appeared to be consistent with the hypothesis that syphilitic infection might induce a population of nonspecific suppressor cells. Subsequent experiments, however, failed to identify either an adherent or nonadherent suppressor cell type (Table 2). Moreover, the addition of normal lymphocytes or adherent cells to cultures of infected cells did not restore reactivity, indicating that depletion of helper T-cells or macrophages was not responsible. These findings led us to question whether immune complexes or antibody bound to the surface membranes of cells might be interfering with the appropriate cellular interactions required for maximal humoral responses to the heterologous antigen. Accordingly, when lymphocytes from syphilitic rabbits were treated with Pronase or trypsin, substances that might remove CIC or bound antibody from membrane surfaces, the immune suppression was abolished (Table 3). Furthermore, cell-free washings obtained from the preparation of infected spleen and lymph node cells were also capable of suppression of the in vitro response of normal primed cells (Table 4).

On the basis of these findings, we then directed our attention to the possible role of CIC in the observed immune suppression; sera from i.v. infected animals were examined for complexes by the Raji cell assay and the Clq-SPA. A strong association was found among (i) overt clinical disease in the experimental host; (ii) the treponemal burden in the involved tissues; (iii) elevated levels of CIC in the sera; and (iv) the ability of these sera to suppress the in vitro immunoglobulin response of normal rabbit lymphocytes. Sera that contained CIC suppressed in vitro immunoglobulin responses, and the greatest degree of suppression was observed in sera that had the highest levels of CIC (Table 6). Overall, these findings appear to be consistent with previous studies which have shown: (i) that CIC may be carried over on spleen cells, thereby altering immune responsiveness (13, 20); and (ii) that CIC are present in active syphilitic infection in the human (23). Additional preliminary studies (R. E. Baughn and D. M. Musher, unpublished observations) have shown that immune complexes, made by reacting syphilis-immune rabbit serum and cardiolipin (VDRL antigen) in antigen excess, but not in antibody excess, can cause distinct suppression of immunoglobulin responses.

The immunosuppressive ability of MPS material, which is abundantly present in syphilitic lesions, infected testes, and the serum of syphilitic rabbits, has been demonstrated recently; MPS was found to suppress the in vitro response of normal rabbit lymphocytes to concanavalin A (4). Although MPS was present in the sera of infected rabbits, no correlation was found in the present study between its presence and the activity of infection or the ability of serum to suppress the immunoglobulin response to SRBC.

The fact that antibodies of various kinds are

present early in syphilitic infection by no means excludes a role for immune suppression. An organism as complex as *T. pallidum* undoubtedly presents a variety of immunogenic determinants to the host. The nature of all treponemal antigens is, as yet, unknown; it is not even known whether VDRL, FTA-Abs, TPHA, or TPI antibodies are directed against the same or different antigenic determinants. Although the importance of these antigens in the diagnosis of the disease seems clear, their role in host resistance is controversial. As no specific, protective immunogens have been identified, one can certainly question the importance of the humoral events associated with the early stages of *T. pallidum* infection.

Although most of our evidence is indirect, the results are in accord with the hypothesis that immune complexes formed at the height of the treponemal burden act to suppress certain IgG responses, a possible explanation for the host's failure to produce an appropriate treponemacidal antibody by the end of the primary stage of syphilis. An antibody with in vivo treponemacidal activity, if indeed one does exist, would be inconsistent with concomitant immunity, however. It seems more plausible that suppression of IgG responses to certain T-dependent antigens might result in failure of the host to develop antibodies which would appropriately act in concert with cellular immunity to limit the growth of the organism. It is not difficult to envision that these events could be occurring at the same time that the host is stimulated to make immunoglobulin to other, perhaps inconsequential, antigenic determinants. Similar states of immunological unresponsiveness have been described for certain protozoan infections in which heightened production of specific antibodies to the parasites occurs in the face of depressed humoral responses to other antigens (5). Experiments are currently under way in our laboratory to characterize the CIC in the sera of rabbits with disseminated infection and determine whether or not they are in fact responsible for the delay in the onset of protective immunity and/or the etiology of lesions in secondary syphilis.

ACKNOWLEDGMENTS

We gratefully acknowledge the technical assistance of Cynthia Adams and Evelyn Heaphy and the secretarial assistance of Mona Thomas.

This work was supported by The Veterans Administration and Public Health Service grants AI-12608, AI 16308 and HD-12247 from the National Institutes of Health.

LITERATURE CITED

1. **Ahlin, T. D., K. S. K. Tung, L. C. Walker, C. D. Leonard, and R. C. Williams, Jr.** 1978. Decrease in circulating immune complexes during hemodialysis. Am. J. Med. **64**:672-677.
2. **Baughn, R. E., and D. M. Musher.** 1978. Altered immune responsiveness associated with experimental syphilis in the rabbit: elevated IgM and depressed IgG responses to sheep erythrocytes. J. Immunol. **120**:1691-1695.
3. **Baughn, R. E., and D. M. Musher.** 1979. Aberrant secondary antibody responses to sheep erythrocytes in rabbits with experimental syphilis. Infect. Immun. **25**: 133-138.
4. **Bey, R. F., R. C. Johnson, and T. J. Fitzgerald.** 1979. Suppression of lymphocyte response to concanavalin A by mucopolysaccharide material from *Treponema pallidum*-infected rabbits. Infect. Immun. **26**:64-69.
5. **Bloom, B. R.** 1979. Games parasites play: how parasites evade immune surveillance. Nature (London) **271**:21-26.
6. **Clark, E. G., and N. Danbolt.** 1964. The Oslo study of the natural course of untreated syphilis. An epidemiologic investigation based on a re-study of the Boeck-Bruusgaard material. Med. Clin. North Am. **48**:612-623.
7. **Hay, F. C., L. J. Nineham, and I. M. Roitt.** 1976. Routine assay for the detection of immune complexes of known immunoglobulin class using solid phase Clq. Clin. Exp. Immunol. **24**:397-400.
8. **Ibrahim, A. N., and M. M. Streitfeld.** 1973. The microassay of hyaluronic acid concentration and hyaluronidase activity by capillary turbidity (CT) and capillary turbidity reduction (CTR) tests. Anal. Biochem. **56**: 428-434.
9. **Jerne, N. K., and A. A. Nordin.** 1963. Plaque formation in agar by single antibody-producing cells. Science **140**: 405-406.
10. **Levene, G., J. Turk, D. Wright, and A. Gimble.** 1969. Reduced lymphocyte transformation due to a plasma factor in patients with active syphilis. Lancet **ii**:246-247.
11. **Levene, G., D. Wright, and J. Turk.** 1971. Cell-mediated immunity and lymphocyte transformation in syphilis. Proc. R. Soc. Med. **64**:14-16.
12. **Ly, I. A., and R. I. Mishell.** 1974. Separation of mouse spleen cells by passage through columns of Sephadex G-10. J. Immunol. Methods **5**:239-247.
13. **Morgan, E. L., and C. H. Tempelis.** 1977. The role of antigen-antibody complexes in mediating immunologic unresponsiveness in the chicken. J. Immunol. **119**:1293-1298.
14. **Moretta, L., S. R. Webb, C. E. Grossi, P. M. Lydyard, and M. D. Cooper.** 1977. Functional analysis of two human T-cell subpopulations: help and suppression of B-cell responses by T cells bearing receptors for IgM or IgG. J. Exp. Med. **146**:184.
15. **Musher, D. M., R. F. Schell, R. H. Jones, and A. M. Jones.** 1975. Lymphocyte transformation in syphilis: an in vitro correlate of immune suppression in vivo? Infect. Immun. **11**:1261-1264.
16. **Musher, D. M., R. F. Schell, and J. M. Knox.** 1974. In vitro lymphocyte response to *Treponema refringens* in human syphilis. Infect. Immun. **9**:654-657.
17. **Pavia, C. S., J. B. Baseman, and J. D. Folds.** 1977. Selective response of lymphocyts from *Treponema pallidum*-infected rabbits to mitogens and *Treponema reiteri*. Infect. Immun. **15**:417-422.
18. **Pavia, C. S., J. D. Folds, and J. B. Baseman.** 1976. Depression of lymphocyte response to concanavalin A in rabbits infected with *Treponema pallidum* (Nichols strain). Infect. Immun. **14**:320-322.
19. **Pavia, C. S., J. D. Folds, and J. B. Baseman.** 1977. Selective in vitro response of thymus-derived lymphocytes from *Treponema pallidum*-infected rabbits. Infect. Immun. **18**:603-611.

20. **Scott, D. W., and B. H. Waksman.** 1969. Mechanisms of immunologic tolerance. I. Induction of tolerance to bovine gammaglobulin by injection of antigen into infant organs *in vitro*. J. Immunol. **102**:347–354.
21. **Setcavage, T. M., and Y. B. Kim.** 1978. Inhibition of the immune response by membrane-bound antibody. J. Immunol. **121**:1706–1710.
22. **Sokal, R. R., and F. J. Rohlf.** 1969. Biometry. The principles and practice of statistics in biological research. W. H. Freeman and Co., San Francisco.
23. **Solling, J., K. Solling, K. U. Jakobsen, and E. From.** 1978. Circulating immune complexes in syphilis. Acta Derm. Venereol. **58**:263–267.
24. **Theofilopoulos, A. N., C. B. Wilson, V. A. Bokisch, and F. J. Dixon.** 1974. Binding of soluble immune complexes to human lymphoblastoid cells. II. Use of Raji cells to detect circulating immune complexes in animal and human sera. J. Exp. Med. **140**:1230–1244.
25. **Theofilopoulas, A. N., C. B. Wilson, and F. J. Dixon.** 1976. The Raji cell radioimmune assay for detecting immune complexes in human sera. J. Clin. Invest. **57**: 169–182.
26. **Ware, J. L., J. D. Folds, and J. B. Baseman.** 1979. Serum of rabbits infected with *Treponema pallidum* (Nichols) inhibits *in vitro* transformation of normal rabbit lymphocytes. Cell. Immunol. **42**:363–372.
27. **Wicher, V., and K. Wicher.** 1977. *In vitro* cell response of *Treponema pallidum*-infected rabbits. I. Lymphocytes transformation. Clin. Exp. Immunol. **24**:480–486.
28. **Wicher, V., and K. Wicher.** 1977. *In vitro* cell response of *Treponema pallidum*-infected rabbits. II. Inhibition of lymphocyte response to phytohaemagglutinin by serum of *T. pallidum*-infected rabbits. Clin. Exp. Immunol. **24**:487–495.
29. **Yonemasu, K., and R. M. Stroud.** 1971. Clq rapid purification method for preparation of monospecific antisera and for biochemical studies. J. Immunol. **106**:304–313.