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Demonstration and Immunochemical Characterization of Natural, Autologous Anti-Idiotypic Antibodies throughout the Course of Experimental Syphilis

ROBERT E. BAUGHN

Departments of Microbiology and Immunology and of Dermatology, Baylor College of Medicine, the Program in Medical Technology and Cytogenetics, The University of Texas School of Allied Health Sciences, and the Syphilis Research Laboratory, Veterans Affairs Medical Center, Houston, Texas 77030*

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Immunoglobulin Gs (IgGs) from serial bleeds obtained 1 week to 5 months after infection of rabbits with *Treponema pallidum* were examined for autologous anti-idiotypic (auto-anti-Id) antibodies. The capacities of IgGs to bind ^{125}I-IgG F(ab')$_2$ anti-*T. pallidum* prepared from earlier bleeds were determined by using radioimmunoassays. Results obtained in these assays suggested that auto-anti-Id IgGs were demonstrable between 60 and 180 days postinfection. Following reinfection, 90 days after the initial infection, auto-anti-Id IgGs peaked 6 to 8 weeks later, even though they were first noted as early as 2 weeks postreinfection. In the reinfection model the auto-anti-Id continued to persist throughout the remaining 3 months of the study. As auto-anti-Id antibodies to anti-fibronectin antibodies were demonstrable also in reinfected animals, our findings raise questions concerning the possible role of the anti-Id in modulating the Id expression of both *T. pallidum*-specific and autoimmune humoral responses.

Autoimmune responses in syphilis have long been inferred from the elicitation of cardiolipin (Wassermann) antibody (37, 57). Recent work (6, 7, 9, 13, 15, 21, 28, 52) has implicated two other ubiquitous host components in the induction of autoimmune responses in syphilis: fibronectin (Fn) and creatine kinase. Autoantibodies to these two endogenous host proteins appear to contribute to both immune complex formation and the lesion histopathology (27, 28). Although tissue destruction alone could favor the induction of autoimmune responses to these host proteins, other explanations are possible. Fn, a molecule capable of interacting with many cell types, has binding sites for collagen, heparin sulfate, fibrinogen, and fibrin as well as for several microorganisms (41). Unlike most bacteria, *Treponema pallidum* binds to the cell-binding domain of the molecule (4, 54). This host protein, through its interaction with virulent treponemes, appears to play a key role in the formation of immune complexes (4, 9, 15). Whereas it is possible that conformational changes resulting from binding render the molecule immunogenic (7, 9, 21), another yet unexplored explanation seems plausible. Drawing upon the recent hypotheses of Cooke et al. (14) and Plotz (40), autoantibodies to host proteins, such as Fn, could arise from perturbations in the idiotypic (Id) network. Conceivably, as diagrammed in Fig. 1, anti-Fn antibodies might in fact be anti-Id antibodies to an anti-treponemal antibody, specifically those directed against the 83-kilodalton Fn-binding protein or receptor of *T. pallidum*.

The network theory of immune regulation, first proposed by Jerne (26), suggests that Id-anti-Id reactions control the response of a host to an antigen either via a positive (enhancing) or negative (suppressing) feedback mechanism. Despite numerous studies which have implicated Id networks in regulation of the immune response to a variety of antigens (reviewed in references 10 and 44), infectious agents (16–20, 22–24, 29–31, 34, 36, 42, 46, 48, 50, 55, 56), and tumors (reviewed in references 32, 34, and 51), the role of anti-Id responses in syphilitic infection has not been explored. The overall objective of the present study was to determine whether naturally occurring autologous anti-Id (auto-anti-Id) antibodies to *T. pallidum*-specific F(ab')$_2$ fragments could be demonstrated at any time during the first 6 months of experimental syphilis or within a comparable period after reinfection. Such experiments were considered prerequisites to undertaking studies designed to assess the role of anti-Id in resistance to reinfection in this model and the impact of the Id network on autoimmune and *T. pallidum*-specific responses in human infection.

MATERIALS AND METHODS

Organisms. The Nichols strain of *T. pallidum* subsp. *pallidum* was propagated by intratesticular passage in New Zealand White male rabbits. Organisms for intravenous infection and for immunologic studies were extracted from infected testes and separated from host tissue contaminants by gradient density centrifugation with 40% Percoll (Pharmacia Fine Chemicals, Piscataway, N.J.) containing 0.1 mM dithiothreitol (25), as previously described (4). Suspensions of *T. pallidum* proteins in phosphate-buffered saline (PBS), pH 7.4, for the preparation of immunosorbent columns and microassays were allowed to stand overnight at 4°C prior to sonication. Solubilized treponemal antigens for sodium dodecyl sulfate-polyacrylamide gel electrophoresis were prepared as previously described (7, 15).

Suspensions of the avirulent spirochete *Treponema phagedenis* biotype Reiter for additional immunosorbent use were prepared by cultivating organisms in Spirolate-brain heart infusion broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 10% normal rabbit serum for 5 to 7 days at 35°C (8). The organisms were harvested by centrifugation at 10,000 × *g* for 25 min, washed four times with PBS, and subsequently adjusted to contain 4 mg of protein per ml (11) prior to sonication.

Staphylococcus aureus Cowan 1 was used in fluid-phase radioimmunoassays (RIA) for the binding and precipitation of immunoglobulin G (IgG). Cells for those studies were

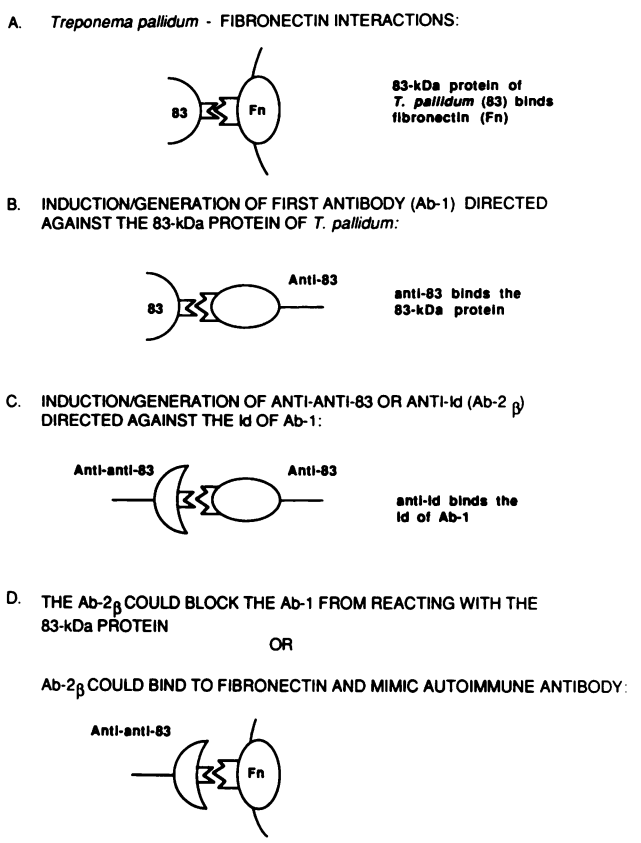

FIG. 1. Figurative explanation of one possible mechanism involving the Id network in secondary syphilis. kDa, Kilodaltons.

grown in tryptic soy broth (BBL) for 18 h, harvested by centrifugation, and suspended in 0.02 M HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) containing 0.1% Formalin. Prior to use, formalinized cells were washed three times in PBS and suspended at a final 10% concentration.

Infection and bleeding of rabbits. Outbred New Zealand White male rabbits were infected intravenously with 4×10^7 *T. pallidum* organisms as described previously (3, 5). Experimental disseminated infection was allowed to progress; the animals were maintained at 18°C, and their backs were shaved at least three times per week. Three animals in which resolution of all lesions was noted by day 85 were rechallenged on day 90 with 4×10^7 organisms. Serum (10-ml) and plasma (25-ml) samples were obtained at regular intervals following infection and reinfection.

Fn-cell attachment fragments (Fn-CAF). The 120-kilodalton domains of human Fn were isolated and purified from α-chymotrypsin proteolytic digestion of plasma according to previously described methods (39, 45). These Fn-CAF were used for the preparation of immunosorbents and in RIA for the assessment of autoimmune responses to Fn following reinfection (described below).

Enzyme-linked immunosorbent assays (ELISAs). Microassays, based on methodologies previously described (6, 8, 9), were used to screen sera for the presence of anti-*T. pallidum* and anti-Fn antibodies. Briefly, polystyrene Immunolon 2 plates (Dynatech Laboratories, Inc., Alexandria, Va.) were coated with 100 μl of Fn-CAF (20 μg/ml) in carbonate buffer (pH 9.6) or with 50 μl of a solubilized suspension of Percoll-purified *T. pallidum* (2×10^6 organisms per ml in PBS). The plates coated with organisms were allowed to dry overnight at 37°C. Then 75 μl of 10% ethanol was added to each well, and the plates were again allowed to dry overnight at 37°C. After nonspecific sites were blocked for 2 h at room temperature with 1% bovine serum albumin (BSA), the plates coated with both types of antigen were washed and 100-μl samples of serially diluted rabbit sera (at 1:50, 1:100, 1:200, and 1:400 initially and then at 1:800 and 1:1,600 if further dilutions were necessary) in PBS containing 1% BSA were added to triplicate wells. The plates were incubated for 2 h at room temperature. Antibody binding was detected with alkaline phosphatase-conjugated goat anti-rabbit IgG, heavy and light chain specific (Tago, Inc., Burlingame, Calif.). Following the addition of phosphatase substrate (Sigma 104; Sigma Chemical Co., St. Louis, Mo.) in diethanolamine buffer and the stopping of all reactions at the end of 45 min by the addition of 3 M NaOH, the optical density (OD) at 405 nm was determined in an automated spectrophotometer. Relative ELISA units were calculated as the product of the OD multiplied by the reciprocal of the endpoint dilution which yielded ODs of 0.2 to 0.6.

Immunosorbents. Solubilized *T. pallidum* and *T. phagedensis* biotype Reiter proteins and Fn-CAF preparations were dialyzed overnight at 4°C against 0.1 M $NaHCO_3$ and cross-linked to cyanogen bromide-activated Sepharose 4B beads (Pharmacia) according to the instructions of the manufacturer.

Purification of IgG F(ab')$_2$ anti-*T. pallidum*. Methods for the purification of F(ab')$_2$ (24, 25) were modified for use with rabbit plasma as follows. A 100-μl volume of 3 M $CaCl_2$ and 100 U of thrombin were added to each 10 ml of plasma and incubated overnight at 4°C. Following removal of the resulting fibrin-containing clots, equal volumes of 100% saturated ammonium sulfate were added slowly to the recalcified plasma. Precipitates which formed at a final saturation of 50% ammonium sulfate were collected by centrifugation at 3,000 rpm (1,500 × *g*) for 30 min at room temperature, dissolved in 0.15 M NaCl, dialyzed against 0.01 M phosphate buffer (pH 6.8), and applied to columns (1 by 10 cm) of DEAE-cellulose equilibrated with the same buffer. The columns were washed with the same buffer, and the eluted materials were finally concentrated to the original starting volumes (10 ml) by ultrafiltration.

The concentrated materials were dialyzed against a buffer of 0.1 M acetate, pH 4.5, and then incubated with pepsin (Sigma) at a 50:1 protein-to-enzyme ratio for 36 h at 37°C. The pHs of the mixtures were adjusted to 8.0 with 2 N NaOH, and the solutions were clarified by centrifugation at 3,000 rpm (1,500 × *g*) for 30 min and finally applied to 7-ml bed volumes of Sephadex G-150 equilibrated with PBS in 20-ml syringes. Materials eluted from the columns were collected in 2-ml fractions, and the ODs at 280 nm were determined. Materials in the second protein peaks which eluted from the columns were collected and concentrated to a final volume of 10 ml. These materials were found to consist exclusively of F(ab')$_2$ fragments of IgG when subjected to Ouchterlony analysis.

Concentrated F(ab')$_2$ fragments from plasma samples, except for those from the preinfection bleeds, were added to immunosorbent columns consisting of 5 ml of Sepharose 4B beads cross-linked to solubilized *T. pallidum* antigens packed in 10-ml syringes. The columns were washed with PBS until fractions of eluates contained less than 1 μg of protein per ml. This F(ab')$_2$ fragments that did not bind to the immunosorbent columns were saved and concentrated as described below for use as controls. The bound materials

were then eluted with 10 ml of 3 M NaCNS (pH 6.0), immediately dialyzed against PBS, and finally concentrated to volumes of approximately 1 ml by embedding the dialysis bags in dry Sephadex G-200. IgG $F(ab')_2$ anti-*T. pallidum* fragments were then absorbed against packed *S. aureus* cells to remove any residual undigested IgG; removal was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by the failure of the materials to bind *S. aureus*.

Iodinations. Ten-microgram amounts of $F(ab')_2$ or IgG were labeled with $Na^{125}I$ (Amersham Corp., Arlington Heights, Ill.), using Iodobeads (35) (Pierce Chemical Co., Rockford, Ill.), to yield specific activities between 2,000 and 4,000 cpm/ng. Goat anti-rabbit IgG(Fc)/7S (Nordic Immunological Laboratories, Capistrano Beach, Calif., and ICN ImmunoBiologicals, Lisle, Ill.) also were iodinated for use in solid-phase assays. Protein-associated and free ^{125}I was separated by using PD-10 columns (Pharmacia) as previously described (7–9). More than 95% of the radioactivity of labeled materials was precipitable with a final concentration of 10% trichloroacetic acid.

Fluid-phase RIA. Binding analyses and studies of competitive inhibition of binding were performed by using fluid-phase RIA principles outlined by other investigators (22, 23, 31, 44). In the fluid-phase assays, 100 μl of ^{125}I-$F(ab')_2$ anti-*T. pallidum* fragments (30,000 to 50,000 cpm, representing 15 to 20 ng) was mixed in Microfuge tubes with 100 μl of IgG (2.5 mg/ml) and incubated for 2 h at 37°C, followed by 18 h at 4°C. At the end of this incubation, 100 μl of a 10% solution of formalinized *S. aureus* Cowan 1 was added and the tubes were incubated at room temperature for 1 h. Following centrifugation (13,300 rpm [14,600 × *g*]), the supernatant fluids were discarded and the *S. aureus* cells were washed three times in PBS containing 1% BSA (RIA grade; Sigma)–0.02% Tween 20. Radioactivity in the Microfuge tubes was counted in an automatic gamma counter. The binding capacity was further assessed by using decreasing concentrations of rabbit IgG (2.5 mg/ml to 25 μg/ml) in the initial step. Nonspecific binding was assessed in parallel tubes receiving only ^{125}I-$F(ab')_2$ anti-*T. pallidum* and *S. aureus*. Other tubes received ^{125}I-$F(ab')_2$ anti-*T. pallidum* and goat anti-rabbit $F(ab')_2$ (ICN ImmunoBiologicals) to determine the total precipitable counts. All determinations were made in triplicate, and all experiments were repeated at least once. To determine whether the same Id determinants were recognized by IgGs obtained at different time intervals, absorption studies were carried out. One-milliliter samples of ^{125}I-$F(ab')_2$ anti-*T. pallidum* (approximately 200 ng) were absorbed with 1 ml of Sepharose 4B alone or with 1 ml of material cross-linked to 25 mg of autologous IgG. Eluates were then evaluated in fluid-phase RIA by using autologous IgG obtained at other time intervals.

Solid-phase RIA. To assess cross-reactivity, a solid-phase assay (31) was modified as follows. Wells of 96-well flexible microdilution plates (Dynatech) were coated overnight at 4°C with purified anti-*T. pallidum* or anti-Fn-CAF $F(ab')_2$ fragments (2.0 μg/ml, 100 μl per well). The wells of control plates were coated with antigen-nonspecific fragments, whereas some wells were left untreated to determine nonspecific counts. The plates were subsequently washed and blocked with 1% BSA in PBS for 1 h. After another series of washes, the rabbit IgG (acid-Id) under test was titrated; 10-fold dilutions were made in 1% BSA in PBS, and 100 μl of each dilution was added to triplicate wells. To assess competitive inhibition of binding by Fn-CAF, additional Id-coated plates were incubated for 2 h at 4°C with various concentrations of inhibitor and washed again prior to the addition of IgG. The plates were incubated for 1 h at room temperature and then washed again. ^{125}I-labeled goat anti-rabbit IgG [Fc specific; absorbed against normal $F(ab')_2$] was diluted in 1% BSA in PBS to yield approximately 5×10^5 cpm/ml, 100-μl volumes were added to each well, and the plates were incubated for an additional hour. After being washed and dried, individual wells were cut out and counted for bound radioactivity.

Statistical methods. Levels of significance (*P* values) were obtained by the two-tailed Student's *t* test (2).

TABLE 1. ELISA titers after infection with *T. pallidum*

Time (day) postinfection	ELISA titer[a] for serum from animal:		
	R7	R8	R10
30	31.2 ± 2.8	10.8 ± 1.6	43.7 ± 5.2
60	167.6 ± 18.4	105.6 ± 8.4	77.2 ± 5.8
90	138.8 ± 16.0	187.6 ± 15.6	117.2 ± 9.0
120	66.8 ± 5.4	101.4 ± 7.2	102.8 ± 9.2
150	75.8 ± 6.2	84.8 ± 7.6	68.6 ± 5.6
180	57.4 ± 6.8	75.6 ± 8.0	50.8 ± 3.8

[a] Relative ELISA units (mean ± standard error of the mean [SEM] of triplicate determinations) calculated as the product of the OD multiplied by the endpoint dilution which yielded ODs of 0.2 to 0.6.

RESULTS

Progression of disease and anti-*T. pallidum* ELISA titers. The progression of experimental disseminated syphilis in each animal was similar to that described previously (3). Briefly, each animal exhibited >80 disseminated lesions over the shaved portion of its back. Lesion development was allowed to progress through necrosis and complete resolution; the latter in all animals was noted by day 115 postinfection. Three of the animals, in which lesion resolution was complete by day 85 postinfection (rabbits anti-Id:R9 [R9], R11, and R12), were rechallenged with 4×10^7 organisms on day 90 and serum and plasma samples for IgG and $F(ab')_2$ fragments, respectively, were collected at regular intervals throughout an additional 20-week period.

Selected serological responses at 30-day intervals for sera from the three animals which were not reinfected are shown in Table 1. IgG-anti-*T. pallidum* titers, expressed in relative ELISA units, peaked 1.5 to 3 months postinfection, thereafter gradually decreasing or leveling off. Responses in the three rabbits which were reinfected on day 90 were quite similar during the first 3 months (data not shown). Amnestic IgG responses with elevated titers peaking between 15 and 45 days postreinfection were characteristic of the reinfection model (data not shown).

Characteristics of $F(ab')_2$ anti-*T. pallidum*-specific fragments. Anti-*T. pallidum*-specific $F(ab')_2$ fragments were isolated from 25-ml volumes of plasma collected from each animal at various time intervals after intravenous infection. The $F(ab')_2$ fragments that bound to the *T. pallidum* immunosorbents (140 to 210 μg) represented approximately 0.12 to 0.23% of the total $F(ab')_2$ materials (80 to 115 mg) applied to the columns. All anti-*T. pallidum*-specific fragments after iodination yielded several specific reactions with *T. pallidum* antigens on Western (immuno-) blots (data not shown), whereas nontreponemal ^{125}I-$F(ab')_2$ failed to react. Following fluorescein conjugation, anti-*T. pallidum*-specific fragments also exhibited strong (+4) fluorescence in fluorescent treponemal antibody tests.

TABLE 2. Capacity of IgG from a *T. pallidum*-infected rabbit to precipitate autologous ^{125}I-F(ab')$_2$ anti-*T. pallidum* obtained earlier in infection[a]

Time of collection (amt, sp act) of ^{125}I-F(ab')$_2$	Mean cpm (% specific binding) for IgG collected at day:			
	90	120	150	180
Preinfection (16 ng, 39,425 cpm)	1,128 ± 137 (3)	552 ± 75 (1)	943 ± 92 (2)	1,143 ± 152 (3)
Day 30 (20 ng, 43,160 cpm)	6,040 ± 918 (14)	3,891 ± 588 (9)	2,460 ± 363 (6)	1,296 ± 134 (3)
Day 60 (17 ng, 36,250 cpm)	4,062 ± 601 (11)	6,294 ± 871 (17)	7,619 ± 1,014 (21)	5,220 ± 653 (14)
Day 90 (15 ng, 34,810 cpm)	1,872 ± 205 (5)	8,357 ± 885 (24)	6,071 ± 639 (18)	6,511 ± 892 (19)

[a] All IgG and F(ab')$_2$ fragments were from a single animal. Experiments were run in triplicate, using constant amounts of rabbit IgG.

Binding of F(ab')$_2$ anti-*T. pallidum* by IgG isolated at different times after infection. Table 2 shows the capacity of serum IgG from a single rabbit (R8) to bind autologous ^{125}I-F(ab')$_2$ anti-*T. pallidum*. IgG obtained 120 and 150 days after infection bound significant amounts of autologous anti-*T. pallidum*-specific F(ab')$_2$ prepared from the 60- and 90-day-postinfection bleedings ($P < 0.05$). These two IgG preparations bound reduced levels of 30-day-postinfection iodinated anti-*T. pallidum*-specific fragments. In contrast, the IgG preparations failed to bind preinfection, non-*T. pallidum* F(ab')$_2$ (Table 2). IgG derived from the bleed on day 90 that served as the source of F(ab')$_2$ anti-*T. pallidum* also failed to exhibit specific binding (Table 2). Figure 2 depicts the results of similar binding studies using 90- and 120-day-postinfection anti-*T. pallidum* F(ab')$_2$ from two other rabbits (R8 and R10). Binding appeared Id specific in that autologous IgG did not bind to radiolabeled, *T. pallidum*-nonreactive fragments (data not shown).

Figure 3 shows the capacity of the three 150-day-postinfection anti-Id IgGs, at various concentrations, to bind 90- (Fig. 3A) and 120-day (Fig. 3B)-postinfection ^{125}I-F(ab')$_2$ anti-*T. pallidum*. In both studies, binding appeared to be saturated when large amounts of IgG (100 μl or 250 μg and 50 μl or 125 μg) were used. The maximal percentage of 90-day-postinfection iodinated fragments bound with 50 μl ranged between 19 and 26%. Rabbit IgG did not bind to the iodinated *T. pallidum*-nonreactive F(ab')$_2$ fragments. Furthermore, preimmune rabbit IgG, as in the other studies, did not bind to the ^{125}I-F(ab')$_2$ anti-*T. pallidum* (data not shown).

Cross-reactivity of postinfection IgGs with anti-*T. pallidum* F(ab')$_2$ fragments. Purified IgGs (anti-Id) from 120- and 150-day-postinfection bleedings were assayed for direct binding to 90-day-postinfection F(ab')$_2$ anti-*T. pallidum* (Id) by solid-phase RIA. Each of the 120-day-postinfection IgG preparations exhibited a large degree of binding for its autologous Id, although cross-reactions were evident (Table 3). IgG from rabbit R7 exhibited considerable cross-reactivity, binding to F(ab')$_2$ fragments from both R8 and R10; binding with fragments from R8 was approximately equal to 10% (compare 2,763 with 2,420). IgGs from animals R8 and R10 showed weaker cross-reactions with the heterologous F(ab')$_2$ fragments. The latter reactions, however, were well above the reactivity with non-*T. pallidum* fragments, which was almost negligible (less than 230 cpm with 10 μg of autologous IgG in each instance; data not shown). Similar cross-reactions also occurred with the 150-day-postinfection IgGs (data not shown).

Binding of F(ab')$_2$ anti-*T. pallidum* by IgGs from different bleeds following reinfection. The capacities of serum IgGs obtained after reinfection to bind autologous ^{125}I-F(ab')$_2$ anti-*T. pallidum* are depicted in Fig. 4. IgGs taken 2 weeks postreinfection and throughout the following 14 weeks bound significant amounts of autologous F(ab')$_2$ anti-*T. pallidum* ($P < 0.05$ for all samples). The binding of ^{125}I-F(ab')$_2$ anti-*T. pallidum* by postreinfection IgGs was significant ($P < 0.05$) in rabbit R11 at the end of the study period, i.e., at 20 weeks. The binding of the radiolabeled Id by autologous postreinfection IgGs was maximal in rabbits R9 and R11 at 6 weeks (37.5 and 30%, respectively) and was maximal in rabbit R12 at 8 weeks (27%). In contrast, IgGs derived from the same serum samples that served as the sources of IgG F(ab')$_2$ anti-*T. pallidum* did not bind autologous ^{125}I-F(ab')$_2$ anti-*T. pallidum* (zero time point). Increasing the quantities of postreinfection IgGs to 500 μg in the assays did not result in increased percentages of autologous F(ab')$_2$ anti-*T. pallidum* bound (data not shown).

Additional experiments were undertaken to determine whether the Id determinants recognized by IgG 2 weeks after rechallenge were contained within the Id determinants recognized later (8 and 12 weeks after reinfection). The results of these experiments are shown in Table 4. In each instance, absorption of iodinated F(ab')$_2$ anti-*T. pallidum* with autologous, insolubilized IgG only partially decreased the capacities of the fragments to bind IgGs obtained at other time intervals. As in the solid-phase assays with IgGs and F(ab')$_2$ fragments obtained after infection (Table 3), cross-reactions with heterologous preparations obtained after reinfection never exceeded 10% of the binding seen in autologous systems (data not shown).

Binding of F(ab')$_2$ anti-Fn by IgG following reinfection. As in earlier studies (9, 28), elevated levels of autoimmune anti-Fn antibodies could be demonstrated in the animals reinfected with *T. pallidum*; ELISA titers in all three animals peaked 14 days after infection at dilutions of 1:200 (data not shwon). By using immunosorbents prepared with Fn-CAF,

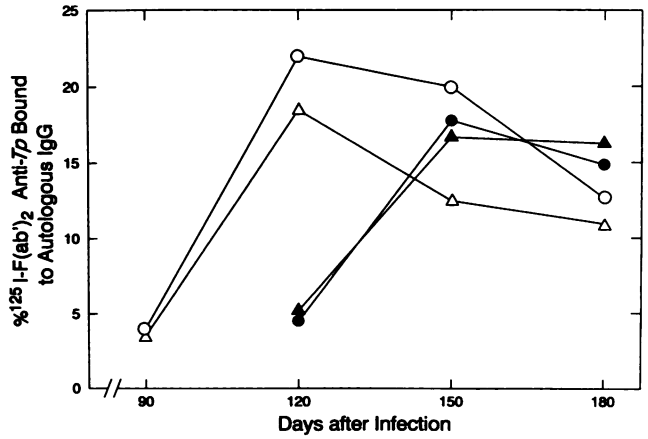

FIG. 2. Binding capacities of autologous, postinfection IgGs to bind anti-*T. pallidum* (anti-*Tp*) F(ab')$_2$ fragments. Binding of 90-day (○) and 120-day (●) fragments by IgG from animal R8 and of 90-day (△) and 120-day (▲) fragments by IgG from animal R10 is shown.

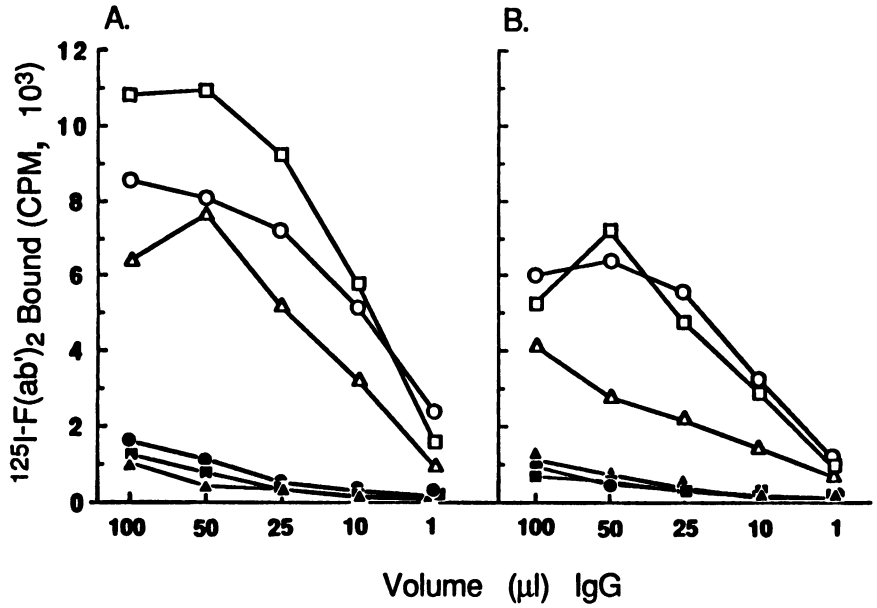

FIG. 3. Binding saturation studies using decreasing concentrations of 150-day-postinfection IgG to precipitate autologous ^{125}I-F(ab')$_2$ anti-*T. pallidum* prepared from bleeds from 90 (A) and 120 (B) days postinfection. Iodinated anti-*T. pallidum* fragments (~20 ng; ~40,000 cpm) were obtained from animals R7 (○), R8 (□), and R10 (△). Iodinated nontreponeme-specific fragments (also ~20 ng; ~40,000 cpm) were from the same bleeds from R7 (●), R8 (■), and R10 (▲).

F(ab')$_2$ anti-Fn fragments were prepared and binding studies were performed with IgG preparations from subsequent bleedings. Serum IgGs obtained 4 weeks after reinfection bound significant amounts of autologous F(ab')$_2$ anti-Fn (Table 5). The binding was maximal at 5 weeks postinfection in rabbits R9 and R12, whereas the response in R11 plateaued between weeks 5 and 7 postreinfection (data not shown).

The purified IgGs from the 28- and 35-day-postreinfection bleedings were assayed for direct binding to each of the anti-Fn F(ab')$_2$ fragments from day 14 postreinfection bleeds in solid-phase RIA. Cross-reactions were noted for IgGs from rabbits R11 and R12 (Table 6); the extent of cross-reactivity with these IgGs approached 10% of that seen with the autologous systems.

In an attempt to determine whether binding of the anti-Id could be inhibited by Fn, the wells of microtiter plates were coated with anti-Fn-CAF and anti-*T. pallidum* F(ab')$_2$ fragments prepared from the 14-day-postreinfection bleedings of animals R11 and R12. Coated and blocked plates were then incubated with increasing concentrations of Fn-CAF or BSA (negative control). Autologous IgGs obtained from both animals 35 days after reinfection were then used to assess inhibition of binding. Preliminary experiments established that it was necessary to first absorb the IgG preparations

TABLE 3. Cross-reactivity of 120-day-postinfection rabbit IgGs with 90-day-postinfection anti-*T. pallidum* F(ab')$_2$

IgG source and concn	Cross-reactivity[a] of anti-*T. pallidum* F(ab')$_2$ fragments from animal:		
	R7	R8	R10
R7			
10 µg	5,264 ± 539	3,017 ± 336	2,629 ± 304
1 µg	4,731 ± 502	2,420 ± 263	1,840 ± 210
100 ng	2,763 ± 318	982 ± 114	763 ± 89
R8			
10 µg	1,578 ± 211	4,247 ± 540	1,394 ± 151
1 µg	749 ± 81	2,596 ± 312	578 ± 66
100 ng	216 ± 32	1,277 ± 161	184 ± 29
R10			
10 µg	1,465 ± 182	2,161 ± 274	3,625 ± 406
1 µg	439 ± 52	952 ± 106	3,421 ± 382
100 ng	175 ± 23	218 ± 29	2,156 ± 239

[a] Mean counts per minute ± SEM of ^{125}I-goat anti-rabbit IgG(Fc)/7S bound; experiments were run in triplicate.

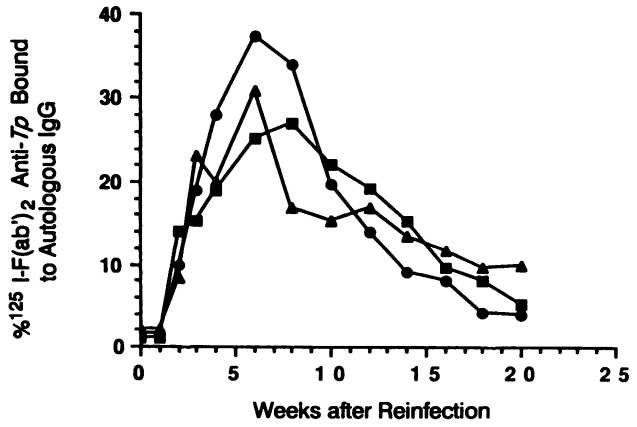

FIG. 4. Binding of ^{125}I-F(ab')$_2$ anti-*T. pallidum* (anti-*Tp*) by autologous IgGs obtained at various time intervals after reinfection. The F(ab')$_2$ fragments were prepared from bleeds on day 90, prior to reinfection. The numbers on the ordinate represent percent binding of ~20 ng (~40,000 cpm) of F(ab')$_2$ from animals R9 (●), R11 (▲), and R12 (■). Binding of nontreponemal ^{125}I-F(ab')$_2$ in each instance was less than 6% (data not shown).

TABLE 4. Effect of absorption of radiolabeled Id with postreinfection IgG on its ability to bind autologous IgG[a]

Source and time (day postinfection) of IgG sample used for binding	Mean cpm ± SEM (% specific binding) of ^{125}I-F(ab')$_2$ anti-*T. pallidum* bound after absorption with autologous IgG from:			
	Preinfection	Day 28	Day 56	Day 84
R9				
28	12,035 ± 1,413	11,842 ± 1,522 (98)	171 ± 24 (1)	4,850 ± 738 (40)	5,442 ± 665 (45)
56	14,613 ± 2,190	14,012 ± 1,921 (98)	11,804 ± 1,776 (81)	184 ± 21 (1)	8,721 ± 1,190 (60)
84	6,027 ± 707	5,963 ± 785 (98)	4,088 ± 445 (68)	1,927 ± 219 (32)	109 ± 14 (2)
R11				
28	10,185 ± 1,306	9,816 ± 1,067 (96)	162 ± 26 (2)	5,236 ± 731 (51)	6,030 ± 878 (59)
56	8,672 ± 1,424	8,269 ± 958 (95)	6,539 ± 937 (75)	140 ± 18 (2)	5,753 ± 697 (66)
84	8,724 ± 1,396	8,575 ± 1,122 (98)	5,321 ± 842 (61)	4,170 ± 532 (48)	133 ± 19 (2)
R12				
28	8,928 ± 1,152	8,594 ± 1,049 (96)	155 ± 24 (2)	5,267 ± 568 (59)	5,694 ± 766 (64)
56	12,683 ± 1,618	12,133 ± 1,772 (96)	10,958 ± 1,702 (86)	174 ± 19 (1)	8,992 ± 962 (71)
84	8,881 ± 1,331	8,657 ± 1,374 (97)	6,374 ± 714 (72)	2,469 ± 333 (28)	163 ± 23 (2)

[a] Experiments were run in triplicate.

used in these studies with human Fn; otherwise no inhibition of binding was seen (data not shown). Free Fn-CAF in both instances inhibited binding of IgG (anti-Id) to the anti-Fn-CAF-specific fragments (Table 7). This inhibition, however, was not complete and appeared to reach a plateau at concentrations of between 1 and 10 μg. Increasing concentrations of BSA, as a negative control, had no effect. Free Fn-CAF and BSA had no effect on the binding of IgG to anti-*T. pallidum*-specific fragments; inhibition was less than 20% (data not shown).

DISCUSSION

This report describes the detection of autologous-anti-Id antibodies as an integral part of the normal immune response of an experimental animal to infection and reinfection with *T. pallidum*. IgG obtained late in infection (120 to 180 days postinfection) was capable of binding autologous F(ab')$_2$ anti-*T. pallidum* derived from earlier bleedings (60 and 90 days postinfection). After reinfection, an accelerated response was seen: the auto-anti-Id appeared to peak earlier and the magnitude of the responses also was much greater. Auto-anti-Id antibodies specific for anti-Fn F(ab')$_2$ also were demonstrable in reinfected animals. Since IgG failed to bind preinfection and nontreponemal F(ab')$_2$, binding in the various assays appeared to be Id specific.

As in other auto-anti-Id studies (12, 19, 22, 38, 43, 44), there are several reasons why binding in the present study may have been underestimated. First, F(ab')$_2$ fragments were prepared only from IgG and not from other immunoglobulin classes. This approach was intentional, so as to avoid IgM-specific Id-anti-Id interactions resulting from the possible sharing of antigen-binding Id determinants between these two immunoglobulin classes (23). Underestimation, especially during the first 2 months, also could have resulted from the inability of the various assays to detect auto-anti-Id bound to Id as a result of immune complex formation. Although sera in the present study were not evaluated for immune complexes, we have previously shown that elevated levels of circulating immune complexes between weeks 2 and 10 of infection are characteristic of the intravenous model (3, 5). Additional factors which may have contributed

TABLE 5. Capacities of IgGs from *T. pallidum*-reinfected rabbits to precipitate ^{125}I-F(ab')$_2$ anti-Fn fragments[a]

Source and time of collection (amt, sp act) of ^{125}I-F(ab')$_2$	Mean cpm ± SEM (% specific binding) of postreinfection IgG collected at day:	
	28	35
R9		
Preinfection (18 ng, 37,520 cpm)	826 ± 142 (2)	719 ± 99 (2)
Day 14 postreinfection (20 ng, 40,350 cpm)	11,294 ± 1,593 (28)	14,932 ± 1,820 (37)
R11		
Preinfection (20 ng, 39,030 cpm)	451 ± 65 (1)	1,134 ± 167 (3)
Day 14 postreinfection (18 ng, 36,760 cpm)	8,457 ± 1,148 (23)	10,734 ± 1,539 (29)
R12		
Preinfection (19 ng, 38,825 cpm)	819 ± 126 (2)	516 ± 90 (1)
Day 14 postreinfection (21 ng, 42,750 cpm)	8,251 ± 1,098 (19)	17,205 ± 2,317 (40)

[a] Autologous IgG and F(ab')$_2$ fragments were tested in triplicate.

TABLE 6. Cross-reactivity of 35-day-postreinfection rabbit IgGs with 14-day-postreinfection anti-Fn F(ab')$_2$

IgG source and concn	Cross-reactivity[a] of anti-Fn F(ab')$_2$ fragments from animal:		
	R9	R11	R12
R9			
10 μg	3,782 ± 431	1,504 ± 192	1,246 ± 153
1 μg	3,387 ± 351	912 ± 116	873 ± 91
100 ng	2,109 ± 216	328 ± 39	265 ± 29
R11			
10 μg	1,212 ± 133	4,839 ± 518	3,265 ± 347
1 μg	534 ± 62	3,422 ± 374	2,193 ± 261
100 ng	173 ± 22	2,653 ± 293	817 ± 94
R12			
10 μg	1,076 ± 116	3,915 ± 403	5,134 ± 538
1 μg	721 ± 83	1,728 ± 195	3,792 ± 365
100 ng	219 ± 24	609 ± 72	1,841 ± 204

[a] Mean counts per minute ± SEM of ^{125}I-goat anti-rabbit IgG(Fc)/7S bound; experiments were run in triplicate.

TABLE 7. Inhibition of anti-Id IgG binding to anti-Fn $F(ab')_2$ by free Fn-CAF

Inhibitor and concn (μg)	Binding[a] (% inhibition) of autologous IgG from animal:	
	R11	R12
None (control)	4,675 ± 523	5,012 ± 561
Fn-CAF		
0.5	3,590 ± 382 (23)	4,150 ± 467 (17)
1.0	2,892 ± 319 (38)	3,341 ± 383 (33)
10.0	2,697 ± 292 (42)	3,053 ± 365 (39)
50.0	2,745 ± 311 (41)	3,156 ± 348 (37)
BSA		
0.5	4,287 ± 578 (8)	4,645 ± 619 (7)
1.0	4,144 ± 529 (11)	4,398 ± 521 (12)
10.0	3,958 ± 503 (15)	4,387 ± 490 (12)
50.0	3,822 ± 526 (18)	4,294 ± 534 (14)

[a] Mean counts per minute ± SEM of ^{125}I-goat anti-rabbit IgG(Fc)/7c bound; experiments were run in triplicate.

to both an underestimation of binding and low levels of cross-reactivity in the present study are as follows: (i) the heterogeneity of the immune response in infected animals; (ii) the fact that the anti-Id at different time intervals may recognize different determinants on $F(ab')_2$ antigen-specific fragments; and (iii) the argument that the immunosorbent methods used perhaps restricted recovery of $F(ab')_2$ fragments to those with the strongest binding affinities.

Early stimulation of the humoral and cellular immune responses is clearly one of the hallmarks of natural and experimental syphilis (reviewed in reference 47). Indeed, the humoral response is quite heterogeneous; specific anti-treponemal antibodies are directed against many different treponemal determinants, irrespective of whether they are major outer membrane antigens. This heterogeneity, the possibility that several different Id systems may exist with respect to any given antigen, and the fact that an individual Id constitutes only a miniscule fraction of the total immunoglobulin which is expressed and recognizes an antigen were seen as distinct disadvantages in attempting to analyze the auto-anti-Id. The major technical constraint imposed by the design of the present study was that our search for anti-Ids was restricted to those reacting against $F(ab')_2$ fragments binding to the *T. pallidum*-immunosorbent. Thus, without intent we were perhaps confined to the Ids on antibodies exhibiting the strongest binding affinities. The only reactions consistently seen when anti-*T. pallidum* $F(ab')_2$ fragments were used for the development of Western blots of *T. pallidum* were against the 47-, 44.5-, 37-, 34-, and 30-kilodalton antigens (data not shown). These results should not be misconstrued as evidence that these polypeptides are either immunodominant or highly immunogenic. Likewise, it does not follow that an auto-anti-Id to the Id of an antibody with the strongest binding affinity would necessarily be the major player in the attempt of the host to down-regulate and restore balance to the immune system following clearance. Clearly, to address such questions and fulfill the second criterion of the network theory (26) much additional work is necessary.

The disadvantages outlined above also undoubtedly impacted with our attempts to determine whether anti-Ids obtained at different intervals postinfection and postreinfection were capable of recognizing overlapping or similar Ids (Table 4) and whether cross-reactions occurred between different animals. Except for animals R11 and R12, the levels of cross-reactivity seen were low or nonexistent. The higher levels of cross-reactive Ids seen between these two animals (Table 6) perhaps reflects the fact that they were siblings; similar findings in members of the same family of outbred rabbits support this suggestion (33).

The studies addressing the question of whether we could demonstrate auto-anit-Ids to anti-Fn antibodies in reinfected animals were much cleaner in design, as they did not involve multiple epitopes on several different antigens. The fact that maximal inhibition by Fn-CAF did not exceed 45% (Table 7), however, suggests that less than one-half of the anti-Id antibodies were directed at idiotopes of the antigen-combining sites. As network controls have been shown to regulate the formation of autoantibodies in other disorders (1, 17–19, 38, 40, 49, 53, 55, 58), it seems plausible that these anti-Ids may represent the attempt of the host to modulate autoimmune Id expression.

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LITERATURE CITED

1. **Abdou, N. I., H. Wall, H. B. Lindsley, J. F. Halsey, and T. Suzuki.** 1981. Network theory in autoimmunity: in vitro suppression of serum anti-DNA antibody binding to DNA by anti-idiotypic antibody in systemic lupus erythematosus. J. Clin. Invest. **67**:1297–1304.
2. **Armitage, P.** 1989. Statistical methods in medical research, p. 99. Blackwell Scientific Publications, Ltd., Oxford.
3. **Baughn, R. E.** 1983. Immunoregulatory effects in experimental syphilis, p. 271–295. *In* R. F. Schell and D. M. Musher (ed.), Pathogenesis and immunology of treponemal infection. Marcel Dekker, Inc., New York.
4. **Baughn, R. E.** 1986. Antibody-independent interactions of fibronectin, C1q, and human neutrophils with *Treponema pallidum*. Infect. Immun. **54**:456–464.
5. **Baughn, R. E., C. B. Adams, and D. M. Musher.** 1983. Circulating immune complexes in experimental syphilis: identification of treponemal antigens and specific antibodies to treponemal antigens in isolated complexes. Infect. Immun. **42**:585–593.
6. **Baughn, R. E., J. L. Jorizzo, C. B. Adams, and D. M. Musher.** 1988. Ig class and IgG subclass responses to *Treponema pallidum* in patients with syphilis. J. Clin. Immunol. **8**:128–139.
7. **Baughn, R. E., M. C. McNeely, J. L. Jorizzo, and D. M. Musher.** 1986. Characterization of the antigenic determinants and host components in immune complexes from patients with secondary syphilis. J. Immunol. **136**:1406–1414.
8. **Baughn, R. E., and D. M. Musher.** 1985. Radioimmunoassays for the detection of antibodies to treponemal polypeptide antigens in serum. J. Clin. Microbiol. **21**:922–929.
9. **Baughn, R. E., V. Wicher, A. Jakubowski, and K. Wicher.** 1987. Humoral response in *Treponema pallidum*-infected guinea pigs. II. Circulating immune complexes and autoimmune responses. J. Immunol. **138**:4435–4440.
10. **Bona, C. A., and H. Kohler.** 1984. Anti-idiotypic antibodies and internal images. Recept. Biochem. Methodol. **4**:141–159.
11. **Bradford, M. M.** 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. **72**:248–254.
12. **Brown, J. C., and L. S. Rodkey.** 1979. Autoregulation of an antibody response via network-induced auto-anti-idiotype. J. Exp. Med. **150**:67–85.
13. **Casavant, C. H., V. Wicher, and K. Wicher.** 1978. Host response to *Treponema pallidum* infection. III. Demonstration of autoantibodies to heart in sera from infected rabbits. Int. Arch. Allergy Appl. Immunol. **56**:171–182.
14. **Cooke, A., R. M. Lydyard, and I. M. Roitt.** 1984. Autoimmunity and idiotypes. Lancet **ii**:723–724.
15. **Dobson, S. R. M., L. H. Taber, and R. E. Baughn.** 1988. Characterization of the components in circulating immune com-

plexes from infants with cogenital syphilis. J. Infect. Dis. **158**:940–947.
16. **Dreesman, G. R., and R. C. Kennedy.** 1985. Anti-idiotypic antibodies: implications of internal image-based vaccine for infectious diseases. J. Infect. Dis. **151**:761–765.
17. **Dumonde, D. C., E. Kasp-Grochowska, J. P. Banga, M. D. Sanders, E. Graham, M. A. Stanford, J. P. Faure, Y. DeKozak, and V. VanTuyen.** 1985. Autoimmune mechanisms in inflammatory eye disease. Trans. Ophthalmol. Soc. U.K. **104**:232–238.
18. **Dumonde, D. C., E. Kasp-Grochowska, E. Graham, M. D. Sanders, J. P. Faure, Y. DeKozak, and V. VanTuyen.** 1982. Antiretinal autoimmunity and circulating immune complexes in patients with retinal vasculitis. Lancet **ii**:787–792.
19. **Dwyer, D. S., R. J. Bradley, C. K. Urquhart, and J. F. Kearney.** 1983. Naturally occurring anti-idiotypic antibodies in myastenia gravis patients. Nature (London) **301**:611–614.
20. **Ertl, H. C. J., and R. W. Finberg.** 1984. Sendai virus-specific T-cell clones: induction of cytolytic T cells by an anti-idiotypic antibody directed against a helper T-cell clone. Proc. Natl. Acad. Sci. USA **81**:2850–2856.
21. **Fitzgerald, T. J., and L. A. Repesh.** 1985. Interactions of fibronectin with *Treponema pallidum*. Genitourin. Med. **61**: 147–155.
22. **Geha, R. S.** 1982. Presence of auto-anti-idiotypic antibody during the normal human immune response to tetanus toxoid antigen. J. Immunol. **129**:139–144.
23. **Geha, R. S., and R. P. Weinberg.** 1978. Anti-idiotypic antisera in man. I. Production and immunochemical characterization of anti-idiotypic antisera to human antitetanus antibodies. J. Immunol. **121**:1516–1523.
24. **Gell, P. G., and P. A. Moss.** 1985. Production of cell-mediated immune response to herpes simplex virus by immunization with anti-idiotypic heteroantisera. J. Gen. Virol. **66**:1801–1804.
25. **Hanff, P. A., S. J. Norris, M. A. Lovett, and J. N. Miller.** 1984. Purification of *Treponema pallidum*, Nichols strain, by Percoll density centrifugation. Sex. Transm. Dis. **11**:275–286.
26. **Jerne, N. K.** 1974. Towards a network theory of the immune system. Ann. Inst. Pasteur Immunol. **125C**:373–389.
27. **Jorizzo, J. L., M. C. McNeely, R. E. Baughn, T. Cavallo, A. R. Solomon, and E. B. Smith.** 1986. Role of circulating immune complexes in human secondary syphilis. J. Infect. Dis. **153**: 1014–1022.
28. **Jorizzo, J. L., M. C. McNeely, R. E. Baughn, T. Cavallo, A. R. Solomon, and E. B. Smith.** 1988. Rabbit model of disseminated syphilis: immunoblot and immunohistologic evidence for a role of specific immune complexes in lesion pathogenesis. J. Cutaneous Pathol. **15**:150–160.
29. **Kaufmann, S. H. E., K. Eichmann, I. Muller, and L. J. Wrazel.** 1985. Vaccination against the intracellular bacterium *Listeria monocytogenes* with a clonotypic antiserum. J. Immunol. **134**: 4123–4127.
30. **Kennedy, R. C., K. Adler-Storthz, R. D. Henkel, Y. Sanchez, J. L. Melnick, and G. R. Dreesman.** 1980. Immune response to hepatitis B surface antigen: enhancement by prior injection of anti-idiotype antibodies. Science **221**:853–855.
31. **Kennedy, R. C., and G. R. Dreesman.** 1983. Common idiotypic determinant associated with human antibodies to hepatitis B surface antigen. J. Immunol. **130**:385–389.
32. **Kennedy, R. C., E. Zhou, R. E. Lanford, T. C. Chanh, and C. A. Bona.** 1987. Possible role of anti-idiotypic antibodies in the induction of tumor immunity. J. Clin. Invest. **80**:1217–1224.
33. **Kindt, T. J., R. K. Seide, V. A. Bokisch, and R. M. Krause.** 1973. Detection of idiotypic cross-reaction among streptococcal antisera from related rabbits. J. Exp. Med. **138**:522–537.
34. **Krolick, K. A., P. C. Isakson, J. W. Uhr, and E. S. Vitetta.** 1979. BCL1, a murine model for chronic lymphocytic leukemia: use of the surface immunoglobulin idiotype for the detection and treatment of tumors. Immunol. Rev. **48**:81–106.
35. **Markwell, M. A.** 1982. A new solid-state reagent to iodinate proteins. I. Conditions for the efficient labeling of anti-serum. Anal. Biochem. **122**:427–432.
36. **McNamara, M. K., R. E. Ward, and H. Kohler.** 1984. Monoclonal idiotypic vaccine against *Streptococcus pneumoniae* infection. Science **226**:1325–1326.
37. **Milgrom, F., and E. Witebsky.** 1962. Autoantibodies and autoimmune diseases. J. Am. Med. Assoc. **181**:706–716.
38. **Nasu, H., D. S. Chia, O. Taniguchi, and E. V. Barnett.** 1982. Characterization of anti-F(ab')$_2$ antibodies in SLE patients: evidence for cross-reacting auto-anti-idiotypic antibodies. Clin. Immunol. Immunopathol. **25**:80–90.
39. **Pierschbacher, M. D., E. G. Hayman, and E. Ruoslahti.** 1981. Location of the cell-attachment site in fibronectin with monoclonal antibodies and proteolytic fragments of the molecule. Cell **26**:259–267.
40. **Plotz, P. H.** 1983. Autoantibodies are anti-idiotype antibodies to antiviral antibodies. Lancet **ii**:824–826.
41. **Proctor, R. A.** 1987. Fibronectin: a brief overview of its structure, function and physiology. Rev. Infect. Dis. **9**:5317–5321.
42. **Reagan, K. J., W. H. Wunner, T. J. Wiktor, and H. Kroprowski.** 1983. Anti-idiotypic antibodies induce neutralizing antibodies to rabies virus glycoprotein. J. Virol. **48**:660–666.
43. **Rodkey, L. S.** 1974. Studies of idiotypic antibodies: production and characterization of autoantiidiotype antisera. J. Exp. Med. **139**:712–720.
44. **Rodkey, L. S.** 1980. Autoregulation of the immune response via idiotype network interaction. Microbiol. Rev. **44**:631–659.
45. **Ruoslahtie, E., G. Hayman, E. Engvall, W. C. Cothran, and W. T. Butler.** 1981. Alignment of biologically active domains in the fibronectin molecule. J. Biol. Chem. **256**:7277–7281.
46. **Sacks, D. L., K. M. Esser, and A. Sher.** 1982. Immunization of mice against African trypanosomiasis using anti-idiotype antibodies. J. Exp. Med. **155**:1108–1119.
47. **Sell, S., and S. J. Norris.** 1983. The biology, pathology, and immunology of syphilis. Int. Rev. Exp. Pathol. **24**:203–276.
48. **Sharpe, A. H., G. N. Gaulton, K. K. McDade, B. N. Fields, and M. I. Greene.** 1984. Syngeneic monoclonal anti-idiotype can induce cellular immunity to reovirus. J. Exp. Med. **160**:1195–1205.
49. **Sikorska, H. M.** 1986. Anti-thyroglobulin anti-idiotypic antibodies in sera of patients with Hashimoto's thyroiditis and Graves' disease. J. Immunol. **137**:3786–3795.
50. **Stein, K. E., and T. Soderstrom.** 1984. Neonatal administration of idiotype or antiidiotype primes for protection against *Escherichia coli* K13 infection in mice. J. Exp. Med. **160**:1001–1011.
51. **Stevenson, G. T., and F. K. Stevenson.** 1983. Treatment of lymphoid tumors with anti-idiotype antibodies. Springer Semin. Immunopathol. **6**:99–115.
52. **Strugnell, R. A., W. F. Williams, G. Raines, J. S. Pedersen, L. P. Drummond, B. H. Toh, and S. Faine.** 1986. Autoantibodies to creatine kinase in rabbits infected with *Treponema pallidum*. J. Immunol. **136**:667–671.
53. **Suleyman, S., D. C. Dumonde, and J. P. Banga.** 1987. Idiotypic expression of antibodies to retinal S-antigen in experimental autoimmune uveoretinitis. Immunology **62**:537–541.
54. **Thomas, D. D., J. B. Baseman, and J. F. Alderete.** 1985. Fibronectin mediates *Treponema pallidum* cytoadherence through recognition of fibronectin cell-binding domain. J. Exp. Med. **161**:514–525.
55. **Tsubouchi, A., K. Yosioka, and S. Kakumu.** 1985. Naturally occurring serum anti-idiotypic antibody against antiliver-specific membrane lipoprotein in patients with hepatitis. Hepatology **5**:752–755.
56. **Uytdehaag, F. G. C. M., and A. D. M. E. Osterhaus.** 1985. Induction of neutralizing antibody in mice against polovirus type II with monoclonal anti-idiotypic antibody. J. Immunol. **134**:1225–1229.
57. **Wasserman, A., A. Neisser, and C. Bruck.** 1906. Eine serodiagnostische Reaktion bei Syphilis. Dtsch. Med. Wochenschr. **32**:745–748.
58. **Zouali, M., and A. Eyquem.** 1983. Idiotypic/anti-idiotypic interactions in systemic lupus erythematosus: demonstration of oscillating levels of anti-DNA autoantibodies and reciprocal antiidiotypic activity in a single patient. Ann. Immunol. (Paris) **134C**:377–391.