

IMMUNIZATION OF MICE AGAINST AFRICAN TRYPANOSOMIASIS USING ANTI-IDIOTYPIC ANTIBODIES

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Treatment with anti-idiotypic (anti-Id)¹ antibodies can, under certain experimental conditions, induce lymphocytes and antibodies of complementary specificity. Injection of minute amounts of anti-Id antibodies has been shown in several experimental systems to induce antigen-specific helper T cells (1–4) and to enhance the expression of the corresponding Id in subsequent antibody responses (5–9). More recently (10), administration of anti-Id has been reported to induce the production of antigen-binding Id-positive molecules in the absence of exposure to antigen. Collectively, these experiments provide clear evidence that idiotypic regulation of the immune system can occur and that antigen-independent mechanisms exist for the expansion of B cell clones bearing the appropriate Id. The application and extension of these findings to the induction of immunity to microbial agents is of obvious interest.

In these studies, we attempted to immunize mice against African trypanosomiasis with anti-Id antibodies. Infection with African trypanosomes, the etiological agents of sleeping sickness, is characterized by a cycling parasitemia, with each cycle consisting of increasing parasitemia, host antibody production, parasite clearance, and appearance of trypanosomes of different variable antigen types (VAT). This antigenic variation apparently occurs spontaneously, with antibody playing a selective role in the elimination of major VAT. It is the ability of the parasite to undergo antigenic variation that is responsible for the cycling parasitemia and the chronicity of the infection (11, 12). We have chosen experimental African trypanosomiasis as a model for anti-Id-induced microbial immunity because (a) immunity is known to be mediated by antibody (13, 14), and (b) protective monoclonal antibodies (Id) can be raised against the variable surface antigens of these parasites. We report here that administration of anti-Id antibodies raised against three such protective Id will induce complete or partial immunity to infection with the homologous clone of *Trypanosoma rhodesiense*. To our knowledge, this is the first demonstration that anti-Id-mediated regulation of Id expression can substitute for antigen in the induction of antimicrobial immunity.

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¹ Abbreviations used in this paper: IBC, idiotype-binding capacity; Id, idiotype; anti-Id, anti-idiotypic; IFA, indirect immunofluorescence assay; NMIG, normal mouse immunoglobulin; PBS, phosphate-buffered saline; RIA, radioimmunoassay; SRBC, sheep erythrocytes; VAT, variable antigen type.

Materials and Methods

Animals. BALB/cJ and SJL/J female mice, 6–8 wk old, were purchased from The Jackson Laboratory, Bar Harbor, ME.

Trypanosomes. The derivation of trypanosome clone WRATat 1.1 from a human isolate of *Trypanosoma brucei rhodesiense* has been described (15). Infection of mice with this clone results in chronic infection, characterized by relapsing peaks of parasitemia and death of the mice between 100 and 150 d. Another clone of *T. rhodesiense*, obtained from Dr. Peter Gardiner, National Institutes of Health, designated EATRO 1886, clone NIHTat 1, bears a VAT that is non-cross-reactive with WRATat 1.1.

Monoclonal Antibodies. The derivation, specificity, and biological activity of monoclonal antibodies 7H11, 11D5, and B7B1 will be described in detail (K. Esser, manuscript in preparation). Briefly, all are derived from fusions of WRATat 1.1 immune spleen cells to the P3/X63-Ag8, IgG₁-secreting myeloma line (16). All are IgG₁, reacting with the VAT of clone WRATat 1.1. In addition, all can neutralize parasite infectivity, as determined by *in vivo* neutralization and passive transfer. The antibodies used for immunization were purified from ascites fluid by affinity chromatography on protein-A Sepharose 4B (Pharmacia Fine Chemicals, Div. of Pharmacia Inc., Piscataway, NJ) and elution with 0.2 M glycine, pH 3.0, 0.5 M NaCl, followed by dialysis against phosphate-buffered saline (PBS).

Preparation and Purification of Anti-Idiotypic Antibodies. SJL mice were immunized by footpad and subcutaneous injections of 75 μ g of purified 7H11, 11D5, or B7B1 protein emulsified with twice the volume of complete Freund's adjuvant (17). The mice were boosted 2 wk later with 75 μ g antigen in incomplete Freund's adjuvant, followed 2 wk later by 75 μ g in saline. Tail bleedings commenced 2 wk after the last boost and were tested for hemagglutination of Id, normal BALB/c IgG (NMIg), or MOPC 21 myeloma protein (IgG₁ κ) coupled to sheep erythrocytes (SRBC), as described (18). Serum from bleedings within each group were pooled and absorbed with normal BALB/c IgG and MOPC 21 coupled to Sepharose to remove activity against heavy chain allotype and MOPC 21 Id. Complete absorption was confirmed by failure of absorbed sera to hemagglutinate NMIg and MOPC 21-coupled SRBC. The IgG₁ fraction of each anti-Id antisera was purified by elution from protein-A Sepharose with 0.1 M sodium citrate, pH 6.0 (19). The IgG₁ fraction of normal SJL sera was similarly prepared. The Id-binding capacity (IBC) of each ¹²⁵I-labeled IgG₁ fraction was determined by calculation of the percent IgG₁ anti-Id completely absorbed by the homologous Id-coupled SRBC preparation. MOPC 21 and NMIg-coupled SRBC were used as control absorbants. It was determined that of the IgG₁ fractions of anti-7H11, -11D5, -B7B1 Id, 11%, 9%, and 6%, respectively, reacted specifically with their homologous idiotypes.

Treatment of Mice with Anti-Id. BALB/c mice, 2–4 mo of age, were treated with various doses, calculated with respect to IBC, of the IgG₁ fractions of each of the three anti-Id. The required concentration of one anti-Id was prepared, pooled with the identical concentration of the other two, and then injected intraperitoneally into mice in 0.5 ml saline. Mice were test bled from tail veins periodically after anti-Id treatment and infection to monitor serum Id levels.

*Infection with *T. rhodesiense*.* Control and anti-Id-treated mice were infected with 100 trypanosomes of clone WRATat 1.1 or NIHTat 1, as described (15). Blood parasitemias were determined daily by examination of wet tail blood smears under high power (400 \times). Counts were converted to trypanosomes per milliliter, using a calibration curve.

Determination of VAT. An indirect immunofluorescence assay (IFA) was used to detect specific VAT of individual trypanosomes in potentially mixed populations of *T. rhodesiense* organisms (20). Trypanosomes appearing during the first peak of parasitemia during WRATat 1.1 infection were examined for VAT using thin smears of trypanosome-infected mouse blood. The antigen slides were air dried, fixed in acetone, and stored at -20°C . Thawed antigen slides, overlaid with individual or pooled anti-WRATat 1.1 monoclonal antibodies, diluted 1/20, plus a 1/80 dilution of a mouse monoclonal antibody specific for a common trypanosome antigen, were incubated in a moist chamber at 37°C for 30 min. The slides were washed in PBS for 5 min, air dried, and overlaid with fluorescein-conjugated goat anti-mouse immunoglobulin (N. L. Cappel Laboratories, Cochranville, PA) diluted 1:40 in PBS. Slides were incubated at 37°C for an additional 30 min, washed, and coverslips applied with pH 7.2 buffered glycerol. Organisms binding monoclonal anti-VAT antibodies exhibited intense,

uniform fluorescence and were readily distinguished from those binding only the monoclonal antibody against the common trypanosome determinant. Detection of the NIHTat 1 VAT was accomplished by an identical method, using a VAT-specific rabbit antiserum and fluorescein-conjugated goat anti-rabbit immunoglobulin.

Radioimmunoassay (RIA). Id concentrations in sera were determined in a solid-phase RIA in which the capacity of serum to inhibit the binding of ^{125}I -labeled Id to the homologous anti-Id was measured (21). Briefly, microwell plates were coated with protein A Sepharose-purified anti-Id (50 μl , 150 $\mu\text{g}/\text{ml}$) for 18 h at 4°C, followed by three washings with PBS. The plates were incubated for 1 h with 50% fetal calf serum in PBS and, after three washings, incubated for 3 h at 4°C with saline, various proteins, or sera either undiluted or diluted 1:10 in saline. After three washings, the homologous Id, labeled with ^{125}I by the chloramine T method (22), was added to each well (50,000 cpm/50 μl) and incubated 3 h at 4°C. The plates were washed at least five times, and each well was counted in a Nuclear-Chicago gamma counter (Nuclear-Chicago Corp., Des Plaines, IL). The assay was standardized with known concentrations of purified cold homologous Id as inhibitors.

Results

Specificity of Anti-Id Antibodies. The binding of radiolabeled monoclonal antibodies by the homologous anti-Id was shown to be restricted in each case to idiotypic determinants by the failure of NMIg and MOPC 21 to inhibit binding (Fig. 1). In addition, the inability in each case of the heterologous Id to inhibit binding indicates that the Id are non-cross-reactive and derived from distinct clones, each of which has specificity for the WRATat 1.1 VAT.

Immunization with Anti-Id Antibodies. After previous studies in which the subclass of anti-Id was found to influence its regulatory effect (1, 23, 24), IgG₁, shown to be capable of priming for Id expression, was purified from each of the three anti-Id antisera as well as from control, normal SJL sera. We chose to administer the three IgG₁ anti-Id together to improve the chances of inducing immunity. In a pilot experiment for these studies, we found that treatment with 100 ng or 10 ng of the three anti-Id 3–4 wk before challenge had no effect on the outcome of infection, whereas 1 μg appeared to induce protection in some animals. In the experiments described here, doses ranging from 250 ng to 4 μg and administered 3 wk before challenge were chosen in an attempt to optimize the conditions required for protection.

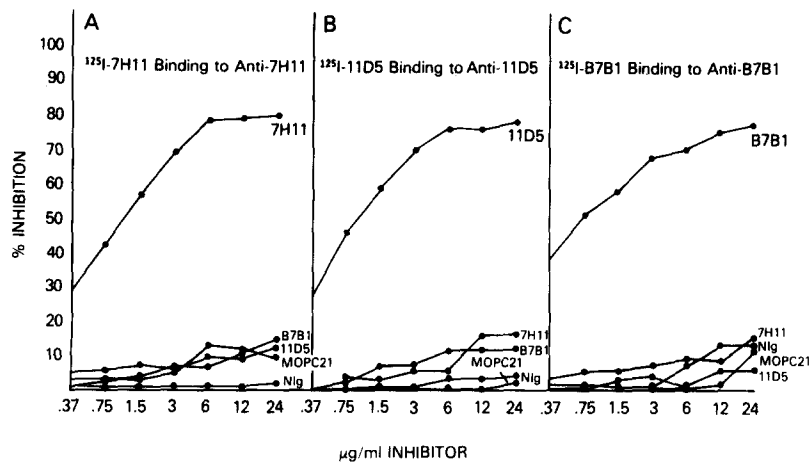


FIG. 1. Inhibition of binding of ^{125}I -labeled Id to the homologous anti-Id by various cold inhibitors.

Infection of BALB/c mice with 100 cloned WRATat 1.1 organisms results in fluctuating waves of blood parasitemia of which 95–100% of the parasites in the first peak bear the original VAT (11). Trypanosomes in the subsequent peaks bear new VAT; therefore, any immunity induced by anti-Id administration will presumptively affect only the first wave parasitemia. We expected the successful induction of a specific anti-VAT response to have three alternative effects on the infection: (a) complete protection, in which the infecting VAT is eliminated before new VAT are able to emerge and no blood parasitemia is ever observed, (b) reduced first wave parasitemia, or (c) selection against parasites bearing the original VAT in favor of different VAT in the first wave, observed as VAT switching. We define parasitemia as reduced when the peak parasitemia in the anti-Id-treated group is less than the lowest peak parasitemia in the controls. In addition, we define VAT switching as having occurred when <10% of the organisms in the first peak bear the original VAT, as defined by reaction with monoclonal reagents in the IFA.

In fact, what we observed were all three effects. As shown in Table I, mice treated with normal SJL IgG₁ at a dose equivalent to the total protein contained in the pooled anti-Id at the 1 μ g IBC concentration had typical primary parasitemias, except for an aberrant mouse in which the original VAT was not expressed. Of the mice treated with either 250 ng, 1 μ g, or 4 μ g of the three anti-Id, within each group were mice that had normal infections, no detectable blood parasitemia, reduced parasitemia, or switched VAT. No obvious dose effect could be appreciated. Considered together, of the 30 mice treated with anti-Id, 63% had altered infections, with complete protection (23%) and VAT switching (27%) being the dominant effects. The first wave parasitemia profiles of the controls (excluding the one aberrant mouse), and the anti-Id-treated mice with reduced parasitemia are shown in Fig. 2. Also included is the course of primary parasitemia of those mice with switched VAT, for which the peak parasitemia was typically delayed by 1 d.

Expression of 7H11 Id Pre- and Postinfection. Sera obtained pre- and post-challenge were analyzed in a competitive RIA for the presence of each of the three Id. In Fig. 3, individual mice are grouped according to the outcome of initial parasitemia. Control mice that received normal SJL IgG₁ and had normal infection had either very low or undetectable levels of the 7H11 Id in their sera, both before and 3 d postinfection (Fig. 3a). The detection of low levels of the 7H11 Id in the majority of mice 2 wk after infection suggests that the Id is normally expressed in response to the first wave parasites that bear the WRATat 1.1 VAT. Of the mice that were treated with anti-Id and had normal infection, there was variable expression of the Id 2 wk

TABLE I
Outcome of Primary Parasitemia in BALB/c Mice Immunized with Anti-Idiotypic Antibodies

Treatment	Normal	Protected	Reduced	Switched	Altered infection/total
Normal SJL IgG ₁	9	0	0	1	1/10
Anti-Id IgG ₁					
250 ng	4	2	1	3	6/10
1 μ m	5	3	1	1	5/10
4 μ m	2	2	2	4	8/10

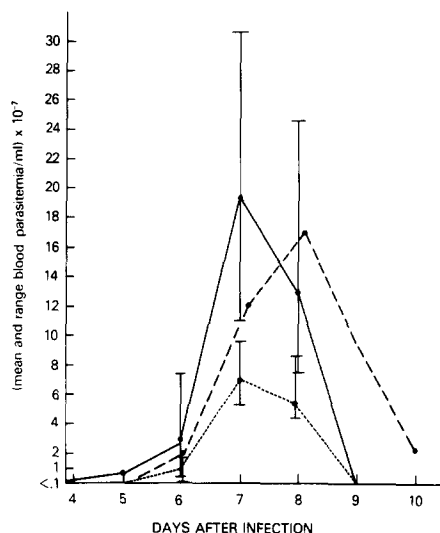


FIG. 2. Mean and range first wave blood parasitemias of WRATat 1.1-infected mice. Controls, normal infection (●—●); anti-Id-treated, reduced parasitemia (●··●); anti-Id-treated, switched VAT, (●---●).

after treatment and before infection. However, all had low or undetectable levels of the 7H11 Id when examined 3 d after challenge. In contrast, relatively high levels of the Id were found 3 d after challenge in all protected mice and all mice that displayed VAT switching. Within the switched group, the only mouse in which this Id was not found at this time was the aberrant control. The 7H11 Id was also found before or early in infection in three of four mice that experienced reduced parasitemia. In all four mice, the levels of Id were highest 2 wk postinfection, subsequent to peak antigen exposure, whereas in the other affected groups in which the concentration of WRATat 1.1 VAT remained minimal, the levels of 7H11 Id had generally declined after 2 wk of infection.

Thus anti-Id administration appeared to affect 7H11 Id expression in the majority of treated mice, and the presence of the Id in high levels shortly after challenge was associated with immunity.

Expression of 11D5 and B7B1 Id. The contribution of the 11D5 Id to the immunity observed is more difficult to ascertain because it is normally expressed earlier and in higher levels in response to WRATat 1.1 infection than the 7H11 Id (Fig. 3b; controls, normal infection). In this case, the sera examined before infection provide the clearest indication of whether the anti-Id treatment affected the expression of the 11D5 Id. This Id was in fact more frequently found, and in higher levels, before infection in the protected and switched groups than in the anti-Id-treated nonimmune mice. Although there were mice within each affected group in which the Id remained silent throughout, it is possible that enhanced expression of both the 7H11 and 11D5 Id mediated the more complete immunity in the majority of mice that were protected. This point is addressed in the experiment to be described in which the anti-7H11 Id was administered alone.

Finally, the B7B1 Id also arose normally in response to WRATat 1.1 infection and generally was detectable early on (Fig. 3c). It did not, however, appear to be readily

inducible by anti-Id, as it was only rarely detected before infection, and then with no greater frequency in the immune than anti-Id-treated nonimmune mice. Thus, there is no evidence that the B7B1 Id contributed to the anti-Id-induced immunity observed.

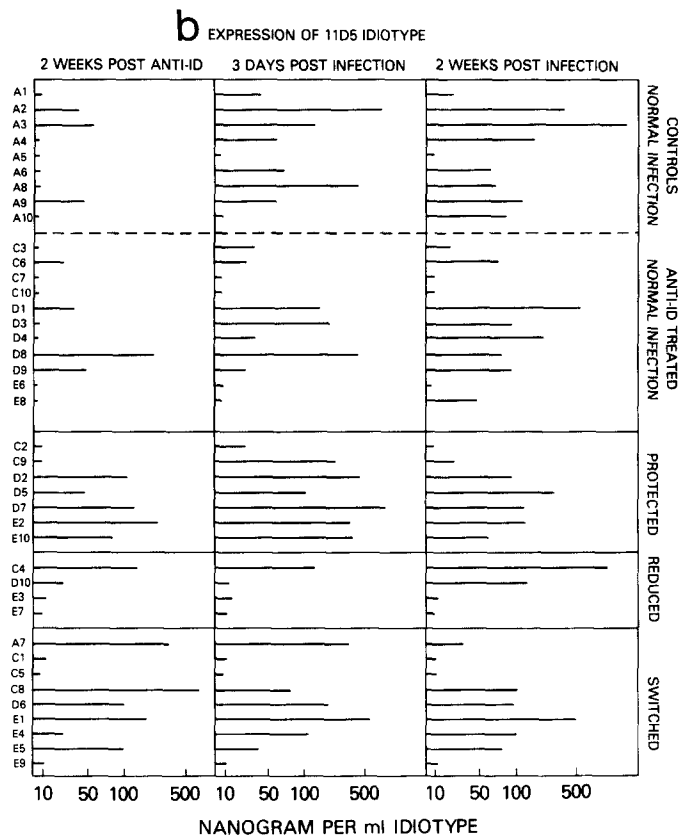
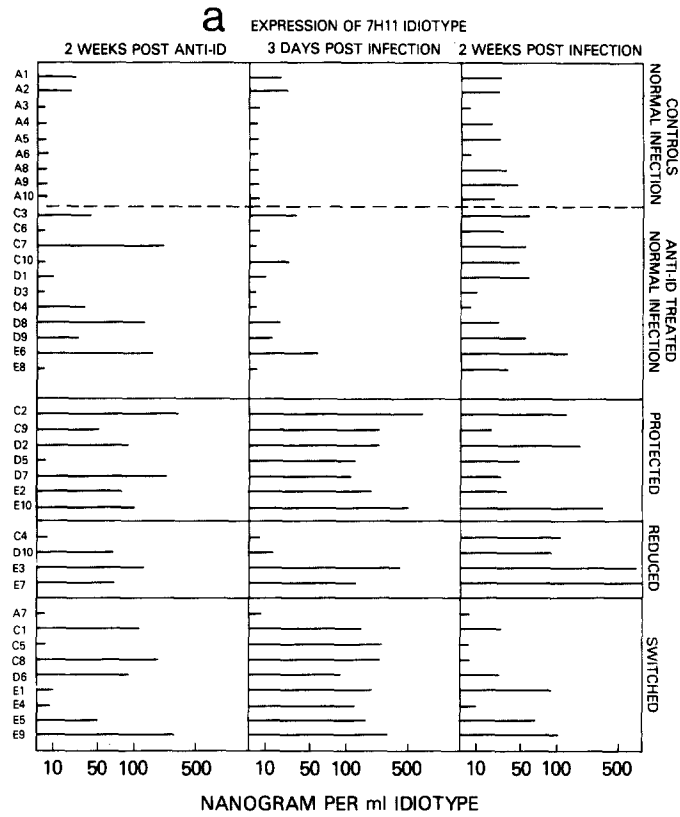
Immunization with Anti-7H11 Id Alone. In this experiment, mice were given 1 μg IBC of the IgG₁ fraction of the anti-7H11 Id or normal SJL IgG₁ followed 2 wk later by an identical dose. Mice were challenged in another 2 wk with either WRATat 1.1, as before, or with a VAT non-cross-reactive *T. rhodesiense* clone NIHTat 1. Of the mice infected with the WRATat 1.1, all 10 control mice had typical infections in which the WRATat 1.1 VAT was expressed on almost all parasites in the first peak. The 7H11 Id was not detected in these mice until 2 wk postinfection, as before (Fig. 4). None of the mice treated with the two doses of anti-Id were completely protected. However, six of nine mice did demonstrate VAT switching, and their peak parasitemias were delayed by a day, as previously observed. In addition, all six mice had detectable 7H11 Id 3 d postinfection (Fig. 4), although the levels were less than those detected in the previous experiment. Thus, the failure to induce complete protection in any of these animals might have been the result of the administration of only the one anti-Id or to the insufficiently high concentration at which the one Id was induced.

Of the mice treated with anti-Id and then challenged with heterologous clone NIHTat 1, none experienced any modulation of their primary parasitemias. This occurred despite enhanced levels of the 7H11 Id in the treated group, either before or 3 d after infection (Fig. 4). The Id was not found in response to NIHTat 1 infection in control mice. Thus, the immunity or partial immunity induced by anti-7H11 Id was specific for the parasite bearing the VAT for which the Id had specificity.

Discussion

In these studies, we demonstrated that administration of anti-VAT anti-Id to mice, in the absence of antigen, can induce immunity against subsequent infection with African trypanosomes bearing the homologous VAT. The immunity is manifested either as complete protection, reduced primary parasitemia, or selection against parasites bearing the original VAT. The immunity appears to be specific in so far as the anti-Id treatment had no effect on the outcome of infection with a heterologous clone of *T. rhodesiense*.

When three anti-VAT anti-Id were administered simultaneously, immunity could be induced in nearly two-thirds of those treated. One Id (7H11) was found selectively 3 d after challenge in animals displaying immunity but was absent in control mice and animals that had been treated with anti-Id but failed to display immunity. Administration of the anti-7H11 Id alone led exclusively to VAT switching in two-thirds of those treated. Again, the effect was associated with the more rapid expression of the 7H11 Id within 3 d after challenge. The effects of the anti-11D5 and B7B1 Id administered alone are currently under study. However, the more frequent detection of the 11D5 Id before challenge in the protected group in particular does suggest that the successful induction of both the 7H11 and 11D5 Id might have been responsible for their complete immunity. The inability to completely protect mice with anti-7H11 Id alone supports this view, although the levels of the 7H11 Id induced in those mice were lower in this case. It is not clear why two doses of the anti-7H11 Id should have been less effective than the one, nor is it clear why the administration of anti-B7B1 Id



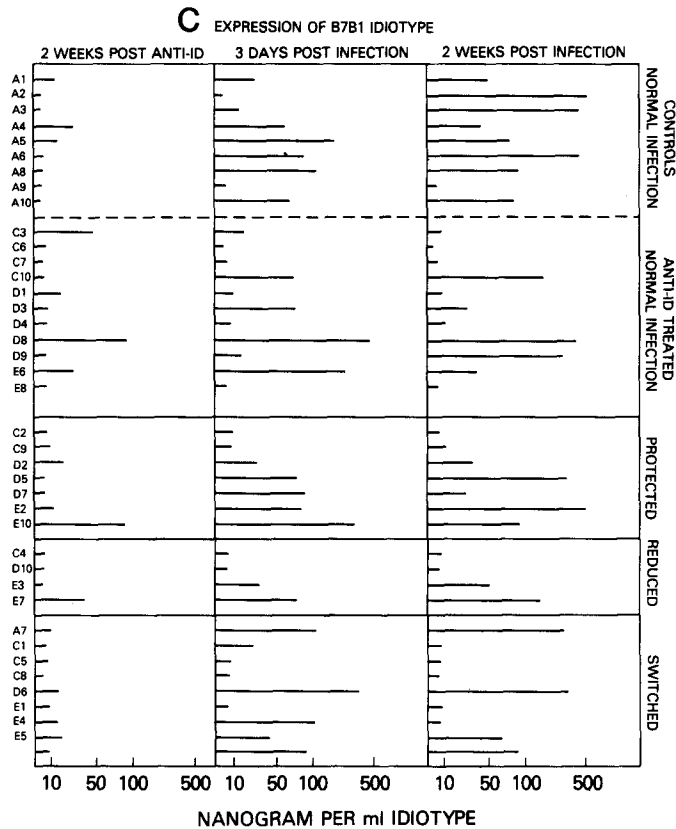


FIG. 3. Expression of individual Id in serum after anti-Id administration and infection. Id concentrations were determined in a competitive RIA standardized with known concentrations of purified homologous Id as inhibitors. Lines represent levels in individual mice grouped according to outcome of primary parasitemia. Group A, normal SJL IgG₁; group C, 250 ng IgG₁ anti-Id; group D, 1 μg IgG₁ anti-Id; group E, 4 μg IgG₁ anti-Id.

in particular should have had so little effect on subsequent B7B1 Id expression. The differences do suggest that the dose and kinetics of anti-Id administration will have to be rigorously explored for each Id whose manipulation is sought to optimize desired biological effects. The source and class of anti-Id might dictate additional refinements or constraints.

Administration of anti-Id antibodies has generally been studied in terms of its effect on Id expression in subsequent immune response to a specified, controlled amount of antigen. This sort of analysis is precluded in these studies because any immunity induced by anti-Id before infection will generally reduce the amount of antigen to which the immune system is subsequently exposed. Thus, immune mice generally develop considerably lower anti-VAT antibody titers after infection than nonimmune mice. We determined Id levels before and early in infection as well as 2 wk after infection, when the anti-VAT antibody response is normally optimum. The induction of antigen binding Id-bearing molecules by anti-Id in the absence of antigen exposure has already been demonstrated by Bluestone et al. (10). We also found Id induced before antigen exposure, and it was more frequently found in mice that then displayed

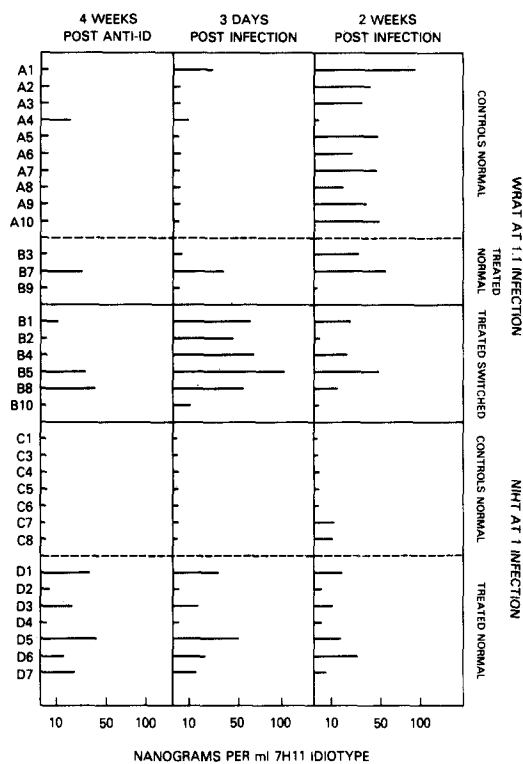


FIG. 4. Expression of 7H11 Id in serum after administration of anti-7H11 Id and infection with WRATat 1.1 or NIHTat 1. Id concentrations were determined as in Fig. 3. Group A, normal SJL IgG₁, WRATat 1.1 infected; group B, IgG₁ anti-7H11 Id, WRATat 1.1 infected; group C, normal SJL IgG₁, NIHTat 1 infected; group D, IgG₁ anti-7H11-Id, NIHTat 1 infected.

some immunity. However, the more striking association appears to have been with 7H11 Id, detectable just after infection, at a time when it had not yet reached detectable levels in infected control mice. Thus, the immunity might not simply have been mediated by pre-existing antibody, but may have also resulted from the rapid activation by antigen of Id-bearing memory clones, established as a result of anti-Id immunization. The higher levels in anti-Id-treated mice of the 7H11 Id, detectable 3 d after WRATat 1.1 infection relative to NIHTat 1 infection, supports this view (Fig. 4).

At present, we are unable to restrict the detection of Id-bearing molecules to those that also have demonstrable antigen-binding activity. Inclusion of parasite antigens in our assays has repeatedly resulted in high nonspecific binding, which obscures detection of antibody below 0.5–1 $\mu\text{g}/\text{ml}$. Therefore, the possibility that the administration of anti-Id has led to the production of Id-bearing nonantigen-binding molecules, as reported elsewhere (8), cannot be excluded. However, this would not explain the association of selective Id expression with immunity.

Can Id induction offer an alternative approach toward immunization against microbial agents in general? We find it encouraging that manipulation of one or two minor idiotypic clones can have a biological effect. Anti-Id immunization could be advantageous in protecting against parasites whose target antigens are difficult to

isolate in sufficient quantity to permit conventional vaccine development. As a practical approach to prevention of African trypanosomiasis, anti-Id-induced immunity to a restricted set of blood form trypanosome VAT is clearly not suitable. The number of different antigen types that the parasites can express are prohibitively large. However, the observation that within the insect vector trypanosomes revert to a limited and relatively constant set of metacyclic VAT suggests that these VAT might be a more hopeful target for Id manipulation (25). Immunization against malaria sporozoites (26) might also benefit from this approach. The recent development of techniques for generating human monoclonal antibodies (27) suggests that in the future monoclonal protective antibodies as well as anti-Id antibodies could be generated from human lymphocytes, thereby allowing the use of isologous immunoglobulins for the induction of Id in man.

Summary

Anti-idiotypic (anti-Id) antibodies were raised against three protective monoclonal antibodies, each with specificity for the variable antigen type (VAT) of a clone of *Trypanosoma rhodesiense*. The IgG₁ fractions of each were pooled and administered to BALB/c mice 3–4 wk before homologous challenge. The course of primary parasitemia was altered in 19 of 30 anti-Id-treated animals. The immunity was manifested as either: (a) complete protection, (b) reduced parasitemia, or (c) selection against parasites bearing the original VAT. The three idiotypes (Id) were found in variable levels in serum during the course of infection in control animals. However, in all anti-Id-treated mice that displayed immunity, one Id in particular (7H11) was detectable much earlier in infection and in higher levels than in control mice or anti-Id-treated, nonimmune mice. Six of nine mice treated with the anti-7H11 Id alone also displayed immunity, manifested in this case exclusively as selection against parasites bearing the original VAT. The effect was again associated with the more rapid appearance of the Id after infection. Specificity of the anti-Id-induced immunity was supported by the failure of anti-7H11 Id treatment to alter the course of infection with a heterologous clone of *T. rhodesiense*. To our knowledge, this is the first report of the antigen-independent induction of antimicrobial immunity using anti-Id antibodies.

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