ficient in calcium may discover that lead relieves some of the symptoms of calcium deficiency. Thus, lead becomes a highly rewarding substance which the child continues to ingest beyond the duration of the calcium deficiency. But lead brings negative symptoms of its own. Why then should pica continue to the point of severe poisoning? Another experiment suggests a possible solution. Calcium-deficient rats were allowed to drink calcium solutions to restore their deficiency. Injections of lithium chloride were then administered concomitantly with calcium ingestion, a procedure that normally induces a long-lasting aversion for the solution it is paired with. However, in the case of calcium-deficient rats drinking calcium, the aversive effects of lithium chloride did not reduce calcium intake (*19*). Possibly for the child with lead pica the reinforcing effects of lead ingestion relieving calcium deficiency are sufficiently powerful that the negative effects of continued ingestion cannot overcome the impetus to ingest lead.

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Antireceptor Antiserum Causes Specific Inhibition of Reactivity to Rat Histocompatibility Antigens

Abstract. *The antigen receptor of lymphocytes destined to form antibody appears to have the characteristics of the immunoglobulin produced. Antibody directed against the combining region of this immunoglobulin should interact with the combining region of the cell receptor for the antigen. Purified Lewis rat alloantibody prepared against Brown Norway (BN) rat histocompatibility antigens was used to immunize L × BN F_1 hybrids. The resultant antiserum has antireceptor activity because (i) it yields precipitin lines in gel diffusion when reacted against the immunizing alloantibody; (ii) it inhibits the hemagglutinin antibody response of Lewis rats to BN histocompatibility antigens; and (iii) it inhibits the local graft-versus-host response of Lewis lymphoid cells against BN̄ antigens. This suggests that antireceptor antibody may inhibit cell-mediated responses as well as antibody responses to histocompatibility antigens and may play a role in the regulation of immune responses to such antigens.*

The interaction of antigen with antigen receptors on lymphoid cells initiates the immune response. Procedures that selectively interfere with this interaction suppress the response. Antibody specific for an antigen can cause selective suppression by preventing antigen from reacting with the receptor (*1*). The antigen receptor on those lymphoid cells that can produce antibody appears to have characteristics of an immunoglobulin (*2*). The combining region of the cell receptor and that of the immunoglobulin produced are presumed to be the same (*3*). Therefore antibody directed against the combining region of an immunoglobulin (anti-idiotypic antibody) should also be directed against the combining region of the corresponding cell receptor. This kind of antibody might therefore suppress the expected antibody response by preventing the interaction of the receptor with the antigen.

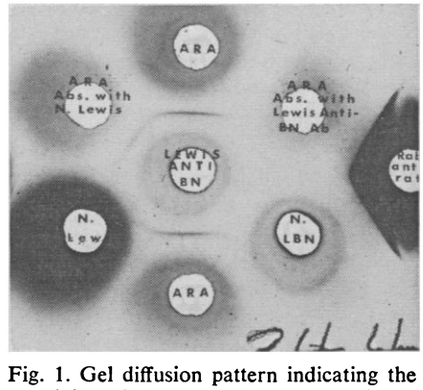

Fig. 1. Gel diffusion pattern indicating the reactivity of ARA. Antireceptor antibody produced in L × BN F_1 hybrids developed precipitin lines with the immunizing antiserum (Lewis alloantiserum to BN), but not with normal Lewis serum, normal LBN serum, or Lewis antiserum to sheep erythrocytes (not shown). Absorption of ARA with Sepharose-conjugated normal Lewis globulin (*ARA abs. with N. Lewis*) did not remove precipitin activity, whereas absorption with Sepharose-conjugated immunizing globulin (*ARA abs. with Lewis anti-BN*) did remove this activity. Sepharose absorption did not remove those immunoglobulins from ARA that were detected by the heterologous rabbit antiserum to rat gamma globulin.

Several observations support this concept (*4*), the most compelling being that presented by Cosenza and Köhler (*5*), who have studied the immune response of the mouse to phosphoryl choline. They have shown that anti-idiotypic antibody reactive with antibody to phosphoryl choline prevented an active immune response to phosphoryl choline when passively administered to intact animals or in cultures of mouse spleen cells before they were exposed to the phosphoryl choline antigen. The response of the mouse to phosphoryl choline is independent of thymus-derived cells, does not result in detectable cell-mediated immunity, and is associated with production of an IgM antibody that is apparently homogeneous.

Antibody to receptor (antireceptor antibody, ARA) could conceivably play a role in the regulation of an immune response. If ARA has such a role, it should be active in controlling immune responses that occur after immunization with complex antigens, as well as in controlling responses to simpler haptenic groups. In order to evaluate these possibilities, the biological effects of a putative ARA prepared against rat alloantibody have been studied.

Fibrosarcoma cells from Brown Norway (BN) rats were lysed in hypotonic solution and centrifuged. The cell membranes were then used to absorb the Lewis alloantiserum (*7*) to BN histocompatibility antigen (37°C for 30 minutes). The absorbed membranes were washed at room temperature,

and the alloantibody was eluted (0.2*M* glycine hydrochloride buffer, *p*H 3.3) (*8*). The hemagglutinin titer of the alloantiserum was 2^{14} and that of the purified antibody (the eluate) was 2^{12} (all corrected to the original volume). The protein content (*9*) of the purified antibody varied from 0.4 to 0.9 mg/ml. The purified antibody was dialyzed and concentrated by lyophilization, and then cross-linked with glutaraldehyde (*10*).

Lewis × Brown Norway (LBN) F_1 adult male rats were immunized with 200 μg of the cross-linked purified alloantibody emulsified in Freund's complete adjuvant. They were immunized again 3 to 5 weeks later with the cross-linked alloantibody emulsified in Freund's incomplete adjuvant. Injections were made subcutaneuosly and intradermally in the anterior thoracic and abdominal walls. Serums were obtained 7 to 10 days after this second injection.

The response of the LBN F_1 animals to immunization with cross-linked alloantibody was assessed in three ways.

1) Serums from immunized LBN animals were tested by gel diffusion against Lewis alloantiserum to BN histocompatibility antigen and against normal Lewis and normal LBN serums.

ARA developed precipitin lines with Lewis alloantiserum (Fig. 1), but not with normal LBN serum, normal Lewis serum, or Lewis antiserum to sheep erythrocytes. Serums from 66 immunized LBN animals were individually tested for precipitin activity in this fashion, and 43 (65 percent) yielded precipitin lines with the Lewis alloantiserum. This precipitin activity could be removed by absorption of ARA with the Sepharose-conjugated globulin fraction of the Lewis alloantiserum, but not by absorption with a similar preparation from Lewis normal serum. The serum from which putative antireceptor antibody had been absorbed reacted with rabbit antibody to rat gamma globulin, indicating that the absorption procedure did not result in overall depletion of immunoglobulin in the absorbed serum. Therefore, the failure of serum absorbed with the globulin fraction of the Lewis alloantiserum to precipitate the Lewis alloantiserum cannot be explained by a generalized depletion of immunoglobulin in the absorbed serum. Since the precipitin lines between ARA and the Lewis alloantiserum do not extend into the wells filled with normal LBN serum or with ARA absorbed with the globulin fraction of the Lewis alloantiserum, it is not possible to determine whether there is any additional reactivity between these serums (*11*). However, antiserum from LBN animals immunized with the Lewis alloantibody can be precipitated with the immunizing antibody and not with normal Lewis serum. These patterns of reactivity cannot be explained on the basis of allotype; it would appear that, at this level of resolution, LBN animals can produce an antibody that reacts with the globulin fraction of the Lewis alloantiserum.

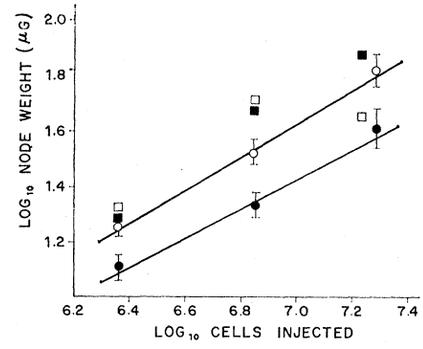

Fig. 2. Local popliteal node graft-versus-host response produced by Lewis and BN spleen cells in L × BN F_1 hosts. The response (the weight of the nodes in milligrams) produced by Lewis spleen cells (●) in L × BN F_1 hosts that had been immunized with purified Lewis alloantibody to BN histocompatibility antigen and possessed ARA demonstrable in gel diffusion was less than that produced by Lewis spleen cells in nonimmunized L × BN F_1 hosts (○) ($P = .02$). The response of BN spleen cells in immunized F_1 hosts (■) did not differ from the BN response in nonimmune hosts (□) ($P = .4$). The responses of Lewis and BN spleen cells in F_1 hosts sham-immunized (see text) were equivalent to the responses observed in nonimmunized hosts ($P > .4$). The points plotted are means ± standard error of mean.

2) The biological effects of ARA were studied in a local graft-versus-host assay (*12*). The hosts were LBN F_1 animals immunized as described above, which were producing ARA detectable in gel diffusion. In this assay, parental aggressor lymphocytes react to the histocompatibility antigens of the other parent as expressed in the F_1 hybrid, and undergo proliferation. If the ARA present in these F_1 hybrid hosts immunized with the Lewis alloantiserum were to play a role in preventing antigen recognition by aggressor lymphocytes, then the reactivity of Lewis parental aggressor cells should be selectively suppressed, whereas the reactivity of BN parental cells should remain unaffected. Uninjected LBN hosts and LBN hosts sham-immunized with Freund's complete adjuvant in saline served as controls. The graft-versus-host activity of BN and Lewis aggressor spleen cells in actively immunized LBN hosts was tested on three separate occasions (Fig. 2). The response of Lewis aggressor spleen cells was suppressed in LBN hosts immunized with the Lewis alloantibody, whereas the response of BN aggressor spleen cells was not affected. The equivalent slopes of Lewis cell reactivity in experimental and control hosts further imply that the inhibition of Lewis graft-versus-host re-

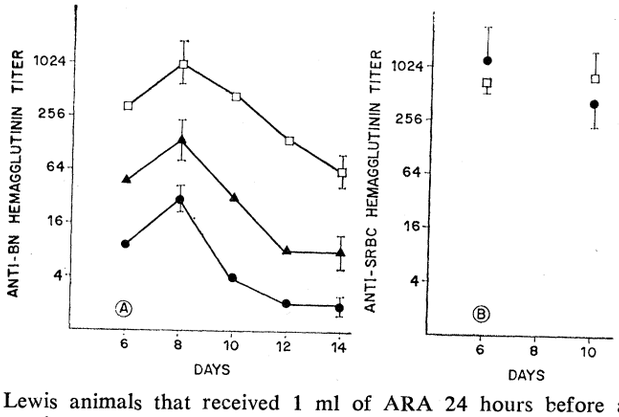

Fig. 3. Suppressive effect of antireceptor antibody on the antibody response to histocompatibility antigens. (A) The hemagglutinin response to BN antigen (anti-BN) by normal adult Lewis rats that had been passively immunized with ARA or normal LBN serum before intraperitoneal injection of 10^7 LBN spleen cells. The response of Lewis animals that received 1 ml of ARA 24 hours before antigen (●) was significantly suppressed ($P < .005$) when compared with that of rats injected with either normal LBN serum (□) or serum from LBN animals injected subcutaneously with the Lewis alloantibody (not shown in figure). The response of Lewis animals that received 1 ml of ARA 1 hour before antigen (▲) was also suppressed ($P < .01$). (B) The production of antibody to sheep red blood cells (anti-SRBC) by other Lewis animals that had been passively immunized with ARA 24 hours before intraperitoneal injection of 10^8 sheep red blood cells (●). This response did not differ from that of rats passively immunized with normal LBN serum 24 hours before injection of sheep RBC (□). The suppression of antibody response by ARA is immunologically specific. The points plotted are means ± standard error of mean.

activity reflects a reduction in the number of effective aggressor cells, rather than an inhibition of some recruitment phase of the response. In the presence of ARA, two to three times as many Lewis aggressor cells are required to achieve the same graft-versus-host response as that observed in control hosts. Therefore, the presence of ARA correlates with a selective antireceptor effect in this assay (*13*).

3) Additional evidence for the antireceptor effect of ARA was obtained by measurements of the hemagglutinin antibody response (*14*) of Lewis animals passively immunized with ARA before receiving an intraperitoneal injection of 10^7 LBN spleen cells. Groups of four or five Lewis animals were injected intravenously with 1 ml of either ARA, normal LBN serum, or serum from LBN animals injected subcutaneously 7 days before exsanguination with 1 ml of the Lewis alloantiserum. This last group of control animals was included in order to evaluate the possibility that passive carry-over of Lewis serum components might be responsible for the biological effects of ARA. In three experiments the effect of ARA on the hemagglutinin antibody response of 53 Lewis rats was measured. The results of one such representative experiment are shown in Fig. 3. Passive immunization with ARA suppressed the hemagglutinin response of Lewis animals to LBN antigens. There is some indication that the effect of ARA given 24 hours before antigen was more pronounced than that produced by injection 1 hour before antigen administration.

Lewis animals were also immunized with 10^8 sheep erythrocytes 24 hours after passive immunization with 1 ml ARA. As shown in Fig. 3B, ARA did not affect the hemagglutinin response of Lewis animals to sheep erythrocytes. The ARA-induced suppression of the hemagglutinin response to BN antigens represents a specific suppression of the immune response.

These results indicate that LBN animals can produce antibody against the Lewis alloantibody, and that this anti-antibody has the properties of an antireceptor antibody. Antireceptor activity is indicated by selective inhibition of Lewis antibody response to BN antigens as well as by inhibition of the response of Lewis lymphoid cells, in the local graft-versus-host reaction against BN antigens. The graft-versus-host response is generally thought to require participation of those thymus-derived lymphoid cells involved in cell-mediated immunity (*15*). It is possible, therefore, that antireceptor antibody may control or inhibit some cell-mediated immune responses. Moreover, recent studies have established that Lewis animals produce an "ARA-like" material upon prolonged immunization with BN antigen (*16*). Thus, ARA may occur naturally in the course of immunization with antigen, and it may well participate in the control or limitation of the Lewis response to BN antigens.

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11. Occasionally, precipitin activity has been observed between normal Lewis serum and certain pools of Lewis alloantiserum. This activity appeared not to be related to the reactivity of the Lewis alloantiserum with ARA. Prolonged heating of fresh normal Lewis serum at 56°C can cause that serum to precipitate with some pools of Lewis alloantiserum. Therefore, we do not heat serums before gel diffusion analysis.
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16. Hyperimmune serums from Lewis animals injected six times with 10^8 x-irradiated BN fibrosarcoma cells at weekly intervals develop a precipitin line in gel diffusion against serum from Lewis animals injected twice with the same tumor cells at an interval of 1 month. The precipitin line between the two Lewis serums formed a line of identity in gel with that line formed between ARA from L × BN F_1 hybrids (see text) and the Lewis antiserum to BN obtained after two antigen injections. Hyperimmune serum did not form precipitin lines with ARA. Thus it would appear that under appropriate conditions the antigen may induce not only an antibody response but also the formation of anti-antibody.
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Neural Pathways from Thalamus Associated with Regulation of Aggressive Behavior

Abstract. *Small electrolytic lesions were made through electrodes in the thalamus of cats at sites where electrical stimulation elicited attack on a rat. Staining by modified Nauta reduced silver methods revealed that significant degeneration passed caudally from the lesions and entered the midbrain dorsal central gray region. Electrical stimulation of this dorsal midbrain region elicited attack on a rat, and destruction of this region suppressed the attack elicited by thalamic stimulation.*

Although medial and midline thalamic structures are involved in the elaboration of aggressive behavior (*1, 2*), little is known of the neural pathways through which such effects on aggressive behavior are mediated. In an attempt to reveal the neural pathways from the thalamus which are involved in the elicitation of aggressive behavior, we made small electrolytic lesions through electrodes in the thalamus of cats at sites where electrical stimulation elicited attack on a rat, and traced the resultant degeneration by use of modifications of Nauta reduced silver staining procedures for degeneration. Since not all of the degeneration after lesion of thalamic attack sites is necessarily related to attack, we also examined the effects on attack of electrical stimulation and lesions of areas suggested by the degeneration to be involved in attack.

The anatomic observations were