cells used for inhibition were added in numbers varying from 10^3 to 10^6 cells. The number of cells that inhibited the lysis of the target cells by 50 percent (35 to 40 percent release of the ^{51}Cr) was determined and used as a criterion for the presence of an antigen associated with acute leukemia. Cell numbers greater than 10^6 were anticomplementary and therefore could not be reliably tested. The inhibition data are summarized in Table 2.

Peripheral white blood cells in numbers from 5×10^4 to 10^6 from each of nine patients with acute lymphocytic or acute myelocytic leukemia with active disease inhibited the cytotoxic activity from the serums and were considered positive. The peripheral white blood cells from three individuals (other than those tested in direct cytotoxicity studies) who were diagnosed as having acute leukemia and who were clinically in remission showed no positive reaction, as judged by the inhibition of cytolysis. Peripheral white blood cells from 17 normal individuals were used, and no inhibition of the cytotoxic reactivity of the antiserums was detected. Bone marrow from two normal individuals was also tested and no inhibition of the antibody activity was found; however, bone marrow from two individuals who had acute lymphocytic or acute myelocytic leukemia inhibited the cytotoxicity of antiserums at cell numbers of 10^4.

To ascertain whether this antigen was an antigen associated with blast cells, peripheral white blood cells from two individuals were exposed to phytohemagglutinin (PHA) for 72 hours. At the end of this time at least 60 percent of the cells were blast cells as determined by morphologic criteria. These PHA-transformed cells did not inhibit the cytotoxic activity of the rabbit serums at 10^6 cells.

It was found that all long-term lymphoid tissue culture cell lines did inhibit this antibody activity. The concentrations of cells needed for inhibition ranged from 2×10^3 to 5×10^4. Two of these cell lines were derived from individuals who were reputedly normal, and seven of the cell lines had been derived from patients with lymphoid malignancies. Two epithelial cell lines, Hela and Hep-2, at concentration of 10^6 did not react with these antiserums. It would appear therefore that tissue culture conditions are probably not responsible for these antigens. The lymphoid tissue culture cells appeared morphologically similar to the blast cells seen in acute lymphocytic or acute myelocytic leukemia. A common antigen on the acute leukemia cells and lymphoid tissue culture cells may reflect similar genetic mechanisms allowing uncontrolled cellular differentiation and proliferation.

It is indeed difficult to establish whether or not differences found between normal and acute leukemic cells represent a quantitative phenomenon. It is technically difficult to obtain the large numbers of cells from patients with leukemia who are in remission and from normal donors needed to do the absorption analysis to establish this point definitively. Our results of the inhibition of cytotoxicity suggest that this antigenic determinant either is present in small quantity or is not exposed on the peripheral white blood cells from the acute leukemic patients in remission, normal peripheral white blood cells, bone marrow, PHA-transformed lymphocytes, and the cells of two epithelial lines.

Of particular interest is the observation that cells from several relatives of the leukemic patients reacted with the antiserums detecting the leukemia-associated antigen or antigens. A similar observation has been made by Morton and Malmgren in studies of cell surface antigens from patients with sarcoma and from their relatives (*5*). This suggests that there may be a common environmental agent inducing the expression of this antigen on the white blood cell surface. The fact that most, if not all, lymphoid tissue culture cell lines contain a demonstrable virus or viral genome may suggest that this antigen is associated with some virus that induces transformation of normal cells into lymphoid or myeloid malignancies. There have been several reports of the presence of viral particles in acute leukemia cells. Whether the antigen detected with these antiserums becomes demonstrable when the cells are infected with this virus remains to be determined.

DEAN L. MANN
G. NICHOLAS ROGENTINE
ROGER HALTERMAN
BRIGID LEVENTHAL
Immunology Branch and Leukemia Service, National Cancer Institute, Bethesda, Maryland 20014

References and Notes

1. A. B. Einstein, D. L. Mann, H. G. Gordon, J. L. Fahey, *Tissue Antigens* **1**, 209 (1971); A. B. Einstein, D. L. Mann, J. L. Fahey, H. G. Gordon, R. J. Trapani, *Transplantation* **12**, 299 (1971).
2. D. L. Mann, G. N. Rogentine, J. L. Fahey, S. G. O. Nathenson, *J. Immunol.* **103**, 282 (1969).
3. Serum from the third rabbit appears to detect the HL-A3 alloantigen.
4. K. R. Mittal, M. R. Mickey, D. P. Singal, P. I. Terasaki, *Transplantation* **6**, 913 (1968).
5. D. L. Morton and R. A. Malmgren, *Science* **162**, 1270 (1968).
6. We thank H. Gordon for technical assistance.

13 August 1971; revised 28 September 1971

Autoimmune Murine Thyroiditis
Relation to Histocompatibility (H-2) Type

Abstract. *Immunization of 33 inbred strains of mice with thyroid extract emulsified in complete Freund's adjuvant showed differences in both thyroid autoantibody response and autoimmune thyroid damage, related to the histocompatibility (H-2) type of the strain. Congenic mice of the same H-2 type exhibited the same pattern of antibody response and thyroiditis, regardless of the strain's genetic background, thus showing a close relation between histocompatibility determinants and autoimmunity.*

Significant differences in the immune responsiveness of different mouse strains to various antigens have been reported (*1*). Lately it has been shown that, in the case of mice immunized with synthetic amino acid polymers, the ability to mount an immune response is under the control of a single autosomal gene, closely linked to the major histocompatibility locus, H-2 (*2*). Gasser described a linkage between the agouti locus and the ability of inbred mice to form antibodies against mouse erythrocyte antigens (Ea-$1^{a,b}$) present only in red cells from wild-type *Mus musculus*. The linkage with the agouti locus implies a linkage with histocompatibility loci H-3 and H-6 (*3*). Susceptibility to various types of murine leukemia was also related to the H-2 locus (*4*).

We know of no correlation thus far reported between histocompatibility type and susceptibility to an autoimmune disease. We report here the existence of a relation between autoimmune thyroiditis of the mouse and the major histocompatibility (H-2) locus. Murine autoimmune thyroiditis is a

convenient model of autoimmune disease, being induced reproducibly by injections of mouse thyroid extract emulsified in complete Freund's adjuvant (*5*). The disease is characterized by circulating thyroid autoantibodies, detectable by different serological methods, and by damage to the target organ; that is, the thyroid which becomes infiltrated with mononuclear cells (*5*).

Female mice, 7 to 8 weeks old, from 33 inbred strains, representing 11 H-2 types, were purchased from Jackson Memorial Laboratories, Bar Harbor, Maine. Ten animals from each strain were injected twice subcutaneously at 7-day intervals with thyroid extract (prepared from randomly bred CF-1 female mice) emulsified in complete Freund's adjuvant (*5*). The animals were killed 4 weeks after the first injection, the time when they usually evince a maximum response with respect to thyroid antibody and pathology. The hemagglutination titer and the pathology index were assessed, as described (*5*).

Table 1. The thyroid antibody response and the thyroid pathology of different inbred mouse strains of 11 H-2 types to thyroid extract. The antibody response is expressed as the mean ± the standard error of the hemagglutination (HA) titer (log_2). The thyroid pathology index (TPI) is expressed as the mean ± S.E. of an arbitrary index. In grade 1, up to 25 percent of the thyroid sections are infiltrated with mononuclear cells; in grade 2, between 25 and 50 percent; in grade 3, between 50 and 75 percent; and in grade 4, more than 75 percent of the thyroid sections are infiltrated. For each strain listed, ten females 7 to 8 weeks old were immunized twice and killed 4 weeks after the first injection.

Strain	H-2 type	HA titer	TPI
A/J	a	9.5 ± 1.0	2.1 ± 0.4
A/HeJ	a	7.9 ± 0.9	2.0 ± 0.3
A/WySn	a	6.9 ± 0.4	2.7 ± 0.3
B10.A	a	11.0 ± 0.4	2.1 ± 0.5
C57L/J	b	7.2 ± 0.8	0.6 ± 0.3
C57BL/6J	b	7.3 ± 0.9	0.5 ± 0.2
C57BL/10J	b	7.5 ± 0.4	0.2 ± 0.05
$C_3H.SW$	b	6.9 ± 0.5	0.3 ± 0.1
129/J	b	6.0 ± 0.9	0.6 ± 0.3
LP/J	b	6.0 ± 0.9	0.5 ± 0.1
BALB/cJ	d	7.8 ± 0.5	0.4 ± 0.1
C57BL/KsJ	d	8.4 ± 0.4	0.8 ± 0.2
DBA/2J	d	5.1 ± 0.9	0.2 ± 0.1
HTG	g	7.4 ± 0.9	0.7 ± 0.3
HTI	i	7.6 ± 0.6	0.3 ± 0.2
AKR/J	k	11.6 ± 0.5	3.3 ± 0.3
B10.BR	k	12.4 ± 0.7	3.3 ± 0.2
CBA/J	k	12.1 ± 0.8	2.7 ± 0.4
C57BR/cdJ	k	12.1 ± 0.5	3.0 ± 0.4
CE/J	k	15.5 ± 0.7	3.0 ± 0.1
C_3H/HeJ	k	11.5 ± 0.5	3.3 ± 0.1
MA/J	k	11.8 ± 0.4	3.1 ± 0.2
RF/J	k	13.8 ± 1.1	3.7 ± 0.1
ST/bJ	k	13.5 ± 0.4	3.7 ± 0.1
AKR.M	m	9.4 ± 0.5	2.2 ± 0.3
BDP/J	p	11.3 ± 0.7	1.0 ± 0.2
BUB/BNJ	q	14.0 ± 0.6	3.0 ± 0.2
DBA/1J	q	12.5 ± 1.4	3.0 ± 0.4
SWR/J	q	11.8 ± 0.7	3.1 ± 0.1
A.SW/J	s	13.2 ± 1.1	3.3 ± 0.3
SJL/J	s	12.5 ± 1.4	3.4 ± 0.2
TN	s	12.5 ± 0.3	3.6 ± 0.1
SM/J	v	3.8 ± 0.7	0.1 ± 0.05

There was a striking correlation between response to thyroid antigen and H-2 type (Table 1). Mice with different H-2 alleles are neither completely susceptible nor completely resistant, but show graded degrees of susceptibility. There is a definite correlation between the antibody response and the thyroid damage—that is, the good responder strains for thyroid autoantibody also responded well with respect to thyroiditis (cellular infiltration of the thyroid). Previous observations have shown that the same strains are good or poor responders to thyroid antigen at any time after the immunization (*6*). For instance, the H-2^s and H-2^k mice were consistently excellent responders, the H-2^q strains were good, the H-2^a strains were fairly good, whereas H-2^b and H-2^d were poor responders, and H-2^v mice responded very poorly.

The responsiveness of the F_1 to thyroid antigen is consistent with an autosomal dominant character (Fig. 1). The F_1 hybrids of BALB/c (H-2^d) and SJL (H-2^s) mice responded as well as the good responder parent to thyroid antigen.

The fact that the response to thyroid antigen is related to the H-2 type and not some other determinant of the strain's genotype is supported by the use of congenic strains for H-2 locus. These strains responded as might be predicted from their H-2 type. The A.SW (H-2^s) mice are congenic with A/WySn (H-2^a); $C_3H.SW$ (H-2^b) are congenic with C_3H/He (H-2^k). The difference in antibody titer between A.SW and A/WySn and $C_3H.SW$ and C_3H/He, respectively, are statistically significant at a $P < .001$. In the unpurified thyroid extract used in our experiment, only thyroglobulin is known to serve as an effective autoantigenic stimulus in rodents (*7*); therefore, a correlation actually exists between the H-2 genotype and the immune response to thyroglobulin. The magnitude of the antibody responses of mice to repeated injections of small doses of a hapten-protein conjugate is also associated with the H-2 type of the strains (*8*). In an attempt to elaborate a general theory of the immune response, Jerne proposed that the antibody genes in the germ line code for antibodies directed against all the major histocompatibility antigens of the species to which the animal belongs (*9*). This may explain why histocompatibility genes influence specific immune responsiveness. A similar conception proposed by Walford holds that all antibodies are directed to histocompatibility antigens or derived from such antibodies (*10*). From this a correlation between some autoimmune diseases, such as systemic lupus erythematosis, and histocompatibility type was postulated. Correlations between the histocompatibility HL-A type and some human diseases have been described (*11*).

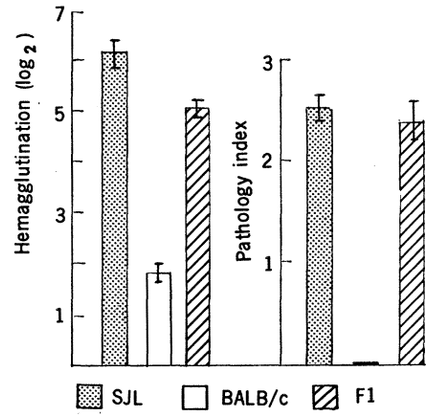

Fig. 1. The immune response of a good (SJL) and poor (BALB/c) thyroid responder strain and of their F_1 hybrid with respect to thyroid antibody and pathology. Eight female animals in each group were immunized subcutaneously twice at 7-day intervals with thyroid extract and complete Freund's adjuvant and killed 2 weeks after the first injection.

In the case of synthetic polypeptide antigens, the ability of the animal to respond could be transferred with "responder's" spleen cells, suggesting a relation to the process of antibody formation (*12*). It would be interesting in our model of autoimmune disease to see what kind of cells could transfer the responsiveness and especially what role the thymus plays in this responsiveness. Further testing has also been undertaken to provide information about linkage of responsiveness, immunologic processing, histocompatibility, and autoimmunity.

ADRIAN O. VLADUTIU
NOEL R. ROSE
Center for Immunology and
Department of Microbiology, State University of New York at Buffalo, Buffalo 14214

References and Notes

1. M. A. Fink and V. A. Quinn, *J. Immunol.* **70**, 61 (1963); N. L. Warner, N. M. Vaz, Z. Ovary, *Immunology* **14**, 725 (1968); W. F. Barth, C. C. McLaughlin, J. L. Fahey, *J. Immunol.* **95**, 781 (1965).
2. H. O. McDevitt and A. Chinitz, *Science* **163**, 1207 (1969).
3. D. L. Gasser, *J. Immunol.* **103**, 66 (1969).
4. J. R. Tennant and G. D. Snell, *Nat. Cancer Inst. Monogr.* **22**, 61 (1966); F. Lilly, *ibid.*, p. 631; F. Lilly, *J. Exp. Med.* **127**, 465 (1968).
5. N. R. Rose, F. J. Twarog, A. Crowle, *J. Immunol.* **106**, 698 (1971).
6. A. O. Vladutiu and N. R. Rose, *Clin. Exp. Immunol.*, in press.
7. N. R. Rose, J. H. Kite, Jr., T. K. Doebbler, R. Spier, F Skelton, E. Witebsky, *Ann. N.Y. Acad. Sci.* **124**, 201 (1965).
8. N. M. Vaz and B. B. Levine, *Science* **168**, 852 (1970).
9. N. K. Jerne, in *Immune Surveillance*, R. T. Smith and M. Landy, Eds. (Academic Press, New York, 1970), p. 3 → *Eur. J. Immunol.* **1**, 1 (1971).
10. R. L. Walford, *Lancet* **1970-II**, 1266 (1970).
11. R. Patel, M. R. Mickey, P. L. Terasaki, *Brit. Med. J.* **2**, 424 (1969); R. L. Walford, S. Finkelstein, R. Neerhout, P. Konrad, E. Shanbarom, *Nature* **255**, 461 (1970).
→ H. O. McDevitt and M. L. Tyan, *J. Exp. Med.* **128**, 1 (1968).

13. Supported by PHS grants CA 05203 and CA 02357. A.O.V. was supported by a Henry C. and Bertha H. Buswell fellowship. We acknowledge the technical assistance of C. Speier and S. Packer. We thank Dr. J. Cruse from the University of Mississippi for supplying TN strain and Dr. D. Schreffler from the University of Michigan for supplying the HTI, HTG, and AKR.M strains.

19 July 1971; revised 24 September 1971 ■

Genetic Control of an Insect Neuronal Network

Abstract. *Motor activity responsible for the calling song of crickets is generated by a small neuronal network whose output is genetically determined. Genes controlling certain output features are located on the X chromosome. The genetic system involved is polygenic and multichromosomal. In some patterns, genetically derived information is adequate to specify the difference of a single impulse in the output of homologous neurons from different genotypes.*

Although animal behavior is directed to varying degrees by genetic information, the way such information is "read out" remains obscure. The nervous system must figure prominently in this process, and many investigations of neurogenetics are appearing (*1*). Progress has been hindered by difficulties dealing with nongenetic factors affecting behavior, in studying small neurons or large populations of neurons, and in finding a circumscribed behavior amenable to both genetic and neurobiological study. Stereotyped insect behavior patterns, such as cricket singing, which are generated by a small network of large, genetically determined neurons, promise to resolve some of these difficulties.

Field crickets produce several songs for intraspecific communication. The best known is the calling song, broadcast by isolated males to attract receptive females. Although bouts of singing are normally triggered by light-dark cycles, crickets have an endogenous singing rhythm and do not require the environmental cue (*2*). The pattern of motor neuron firing responsible for the calling song is generated by a small group of neurons in two thoracic ganglia. Even when isolated from sensory input, this network can produce a song pattern that is indistinguishable from the normal pattern (*3, 4*). Interneurons and motor neurons involved can be monitored, driven, and filled with dye via intracellular microelectrodes (*4*). Similar neuronal programming circuits control many invertebrate behavior patterns (*5*). Among such circuits, the calling song network is unusual in its degree of independence from sensory input and in the resultant invariability of output.

Since the pattern is generated without reference to the current environment of the animal, the network must be using information previously available. The circuit appears to be sequentially laid down during the latter half of the nymphal life-span and is completed before the final molt to adulthood (*6*). To investigate the contribution of genetic information to this process, cricket species with different songs were hybridized, and the sound pulse and motor unit firing patterns of subsequent generations were analyzed. [Work on sound pulses of the wild type and F_1 was done in collaboration with R. Hoy (*7*).] The results show that (i) the song programming network is under firm genetic control and is buffered from variation in the environment; (ii) the genetic system involved is polygenic and multichromosomal, even for single song features; (iii) genes controlling some features are on the X chromosome, while other features are under autosomal control; and (iv) the precision of genetic control is adequate to specify a difference of a single impulse in the trill patterns of identified homologous motor neurons from different genotypes.

Teleogryllus commodus and *T. oceanicus*, Australian and Polynesian field crickets, produce complex calling songs containing a series of chirps and trills arranged in a repeating phrase (Fig. 1, A and F). Females produce 1500 to 2000 eggs (*2*), and the generation time is about 6 weeks at 35°C. The crickets were hybridized by reciprocal crosses (each species was the maternal parent in one cross and the paternal parent in a second cross). Several hundred first filial generation (F_1) nymphs were raised and crossed with both parental species (F_1 females were sterile). Calling songs (at 24.5° ± 1°C) of wild-type, F_1, and backcross males were recorded on tape and filmed on oscillograph paper (Fig. 1). Several hundred consecutive interpulse intervals (time from onset of one sound pulse to onset of the next) were measured, displayed in histograms and successive interval plots, and statistically characterized (Table 1). Eighteen neuronally determined characteristics of the calling pattern were measured in wild-type and F_1 songs; in the backcrosses, all of these features were scanned visually but only three were treated statistically. Except for two backcross classes (Table 1), conclusions were drawn from ten individuals of each type.

The interval structure of wild-type and hybrid calling song patterns demonstrates that this neuronally generated behavior is determined almost exclusively by genotype. Individuals with different genotypes produced different songs that formed a series of patterns bridging the two wild types (Fig. 1, B–E; Table 1). Despite being raised under different conditions of temperature, diet, light cycle, time of year, and population density, individuals always produced calling patterns corresponding to genotype. The "correct" song for a genotype was produced even if an animal was the first of its type to mature and therefore had heard many "incorrect" songs, but none of its own. Individuals with different genotypes produced different song patterns even if raised under nearly identical environmental conditions. These results agree with earlier observations on acoustical behavior of hybrids (*8*). The ultimate source of information for this programming network appears to be genetic, and this will probably be true for similar neuronal programs.

The number of genes or linkage groups that control the network can be estimated by a classical genetic analysis of the number of classes of backcross individuals. [All F_1 charac-