incidence of this syndrome has not been reported to be increased in cretins. Nevertheless, our observations suggest that a possible relationship between thyroid function and respiratory distress syndrome requires further investigation.

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References and Notes

1. R. A. Redding, C. T. Hauck, J. M. Steim, M. Stein, *N. Engl. J. Med.* **280**, 1298 (1969).
2. M. E. Avery, *The Lung and Its Disorders. in the Newborn Infant* (Saunders, Philadelphia, 1968), pp. 170–172.
3. J. M. Steim, R. A. Redding, C. T. Hauck, M. Stein, *Biochem. Biophys. Res. Commun.* **34**, 434 (1969); R. A. Redding, W. H. J. Douglas, K. R. Wunschel, M. Stein, *Fed. Proc.* **30**, 619 (1971).
4. K. Bensch, K. Schaefer, M. E. Avery, *Science* **145**, 1318 (1964); G. B. Dermer, *J. Ultrastruct. Res.* **33**, 306 (1970); S. Buckingham and M. E. Avery, *Nature* **193**, 688 (1962).
5. A. H. Niden, *Science* **158**, 1323 (1967); E. Cuty and P. E. Conen, *Amer. J. Pathol.* **62**, 127 (1971).
6. P. S. Chen, T. Y. Toribara, H. Warner, *Anal. Chem.* **28**, 1756 (1956).
7. T. H. Shepard, *J. Clin. Endocrinol.* **27**, 945 (1967).
8. C. Osorio and N. B. Myant, *Clin. Sci.* **23**, 277 (1962); E. B. Man, M. Adelman, W. S. Jones, R. M. Lord, *Amer. J. Dis. Child.* **119**, 298 (1970).
9. D. Fisher, *Pediatrics* **46**, 208 (1970).
10. ———, *ibid.* **44**, 526 (1969).
11. M. A. Campiche, A. Gautier, E. I. Hernandez, A. Raymond, *ibid.* **32**, 976 (1963); M. E. Avery and J. Mead, *Amer. J. Dis. Child.* **97**, 517 (1959); E. O. R. Reynolds, M. M. Orzalesi, M. M. Motoyama, J. M. Craig, C. D. Cook, *Acta Paediat. Scand.* **54**, 511 (1965).
12. B. Wu, Y. Kikkawa, M. M. Orzalesi, E. K. Motoyama, M. Kaibara, C. J. Zigas, C. D. Cook, *Physiologist* **14**, 253 (1971).
13. We thank K. R. Wunschell, Miss A. B. Munder, and Miss J. Overs for technical assistance. Supported in part by NIHL grant HE 13461-01 and OH-00231.

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Tolerance to Sheep Red Cells: Breakage with Thymocytes and Horse Red Cells

Abstract. *Mice rendered tolerant to sheep red cells and then given normal thymocytes, made no antibody when immunized with these cells. When immunized with horse red blood cells, however, they made significant amounts of noncross-reacting antibody to sheep red blood cells. This suggests that antibody-making precursor cells (B cells) which are nontolerant but nonactivatable by specific antigen, may exist in tolerant hosts.*

The discovery that two types of lymphocytes cooperate in the immune response to certain antigens (*1*) raised the question of where the cellular basis of immunological tolerance lay. It appears that cells in the thymus can easily be rendered tolerant (*2–4*). There is also evidence that cells which have been educated in the thymus and have subsequently migrated to peripheral lymphoid tissues (T cells) can also be rendered tolerant (*5, 6*). The situation with antibody-making precursor cells (B cells), both those in the bone marrow and emigrés in peripheral tissues, is less clear. While several workers have evidence that bone marrow cells of tolerant animals are tolerant (*4, 7*) others have been unable to confirm this (*3, 6*). Even when tolerance was demonstrated in cells in the bone marrow, it was much shorter lived than the tolerant state of the intact animal (*8*). There is no information available that bears directly on the question of whether peripheralized B cells can be rendered tolerant.

Thus it is possible that the tolerant state is a property mediated largely if not exclusively by T cells. This hypothesis has been discussed at length (*9*). Supporting evidence is as follows.

1) Precursor cells competent to make antibodies against "tolerated" antigens have been shown to exist in unresponsive mice; they can only be stimulated, however, by cross-reacting antigens (*10*). Administration of the tolerated antigen with the cross-reacting antigen may prevent the B cell stimulation (*11*).

2) Allogeneic T cells, which can replace the contribution of carrier-specific syngeneic cells (also T cells) in antibody responses to haptens (*12*) allow rats tolerant to sheep red blood cells (SRBC) to make antibodies to SRBC, while syngeneic T cells do not (*13*).

These results are consistent with the notion that B cells of tolerant mice may be normal, that tolerant T cells actively prevent antibody formation, and that some types of nonspecific T cell stimulation can activate the otherwise unresponsive B cells. Our report supports this concept.

Male CBA mice were made tolerant to SRBC as described (*14*). Seven-week-old mice were thymectomized, lethally irradiated (850 roentgens), and then given 5×10^6 syngeneic bone marrow cells and 15×10^6 syngeneic thymocytes. After reconstitution they were given a total of 2.5×10^{10} SRBC intraperitoneally in nine doses, divided over 30 days. Four days after the last injection, serums were titrated, and those mice that had made antibody were eliminated from these experiments. Half of the remaining mice were then given a second inoculation of 15×10^6 syngeneic thymocytes; half of these mice, as well as half of the mice that

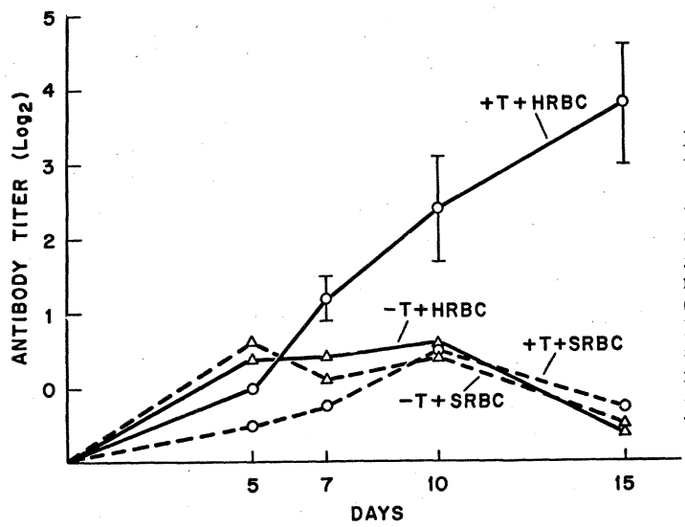

Fig. 1. Antibody to SRBC response of mice rendered tolerant to SRBC and then either given 15×10^6 normal thymocytes (+T) or not (−T). Half of each resultant group was immunized with SRBC and half with HRBC.

did not get the second thymocyte inoculation, were immunized with SRBC and the other half of both groups were immunized with horse red blood cells (HRBC). Figure 1 gives the mean titers of antibody to SRBC in the four groups of mice. The only group that made a significant antibody response to SRBC was the one in which the mice were given normal thymocytes after tolerance induction and immunized with HRBC. Mice that received thymocytes and SRBC responded no better than the mice that were not given thymocytes; these mice failed to respond when immunized with either SRBC or HRBC. Thus, tolerance to SRBC was produced and could be broken only if mice were given an inoculation of normal thymocytes, and then only if immunized with another kind of heterologous red cell (in this case HRBC).

The foregoing observation has been substantiated in three additional experiments, which have also shown the following: (i) The antibodies to SRBC made after immunization with HRBC are not cross-reacting; they were completely removed by one absorption with an equal volume of packed SRBC while their titer was unaffected by four absorptions with HRBC. The absorption with HRBC, however, removed all the agglutinins for HRBC. (ii) The addition of an equal amount of SRBC to the HRBC inoculum not only does not prevent the formation of antibodies to SRBC, but in one experiment boosted the antibody response to SRBC from a peak mean titer of 2.4 ± 0.7 to 4.4 ± 0.4 ($\log_2 2 \pm$ S.E.). (iii) The addition of thymocytes without HRBC is insufficient to break tolerance. (iv) When the tolerant spleen cells are adoptively transferred along with normal thymocytes to syngeneic thymus-deprived mice these secondary recipients make no antibody to SRBC when immunized with HRBC (three separate experiments) although they make a vigorous antibody response to HRBC. The details of the experiments with adoptively transferred cells have been described (*15*).

We offer the following explanation for these results. The multiple SRBC injections rendered the mice tolerant. The added normal thymocytes could not break the tolerance as they were themselves rendered tolerant. [There is direct evidence that normal cells can be rendered tolerant by tolerant cells (*15*).] However, HRBC could stimulate these cells. In a manner which is not entirely clear, the SRBC that remained from the tolerance-inducing injections were able to capture the T cell stimulatory effect produced by the HRBC. Hartmann has described special in vitro conditions where SRBC B cell precursors can capture the effect produced when HRBC stimulate T cells, but only if SRBC are present (*16*). The importance of the residual SRBC antigen in our studies is emphasized by the fact that the HRBC could not break the tolerance of the cells after transfer to a second host and also by the boosting effect produced by the inclusion of SRBC with the HRBC.

Our method of breaking of tolerance differs from previous reports of breaking tolerance with cross-reacting antigens in that the antibodies made did not cross-react with the antigen used for tolerance abrogation (*11, 17*). This shows that specific, as well as cross-reacting B cells, may exist in a nontolerant state in ostensibly tolerant hosts. It also emphasizes the need for T cell activation, as do the above-mentioned experiments with allogeneic thymocytes (*13*), for breakage to occur. Our results differ, however, from those with allogeneic cells in that no immunologic attack, either graft-versus-host or host-versus-graft, is being mounted on B cells which might result in some fundamental changes in that population. Both results, as well as others demonstrating the allogeneic effect, emphasize that at least some parts of the T-B cell interaction may be nonspecific.

The main point we would like to make, however, is that situations may exist where mice are incapable of responding to SRBC, even after the addition of normal T cells and yet still possess significant numbers of nontolerant B cell precursors that can make antibody to SRBC.

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References and Notes

1. J. F. A. P. Miller and G. F. Mitchell, *Transplant. Rev.* **1**, 3 (1969); A. J. S. Davies, *ibid.*, p. 43; H. N. Claman and E. A. Chaperon, *ibid.*, p. 92.
2. K. Isakovic, S. S. Smith, B. H. Waksman, *J. Exp. Med.* **122**, 1103 (1965).
3. R. B. Taylor, *Transplant. Rev.* **1**, 114 (1969).
4. J. M. Chiller, G. S. Habicht, W. O. Weigle, *Proc. Nat. Acad. Sci. U.S.* **65**, 551 (1970).
5. R. K. Gershon, V. Wallis, A. J. S. Davies, E. Leuchars, *Nature* **218**, 381 (1968).
6. J. F. A. P. Miller and G. F. Mitchell, *J. Exp. Med.* **131**, 675 (1970); A. Many and R. S. Schwartz, *Proc. Soc. Exp. Biol.* **133**, 754 (1970).
7. J. H. L. Playfair, *Nature* **222**, 882 (1969).
8. J. M. Chiller, G. S. Habicht, W. O. Weigle, *Science* **171**, 813 (1971).
9. A. C. Allison, A. M. Denman, R. D. Barnes, *Lancet* **1971-II**, 135 (1971).
10. W. O. Weigle, *J. Exp. Med.* **116**, 913 (1962); B. Cinnader and J. M. Dubert, *Brit. J. Exp. Pathol.* **36**, 515 (1955).
11. W. O. Weigle, *J. Immunol.* **92**, 791 (1964).
12. D. H. Katz, W. E. Paul, E. A. Goidl, B. Benacerraf, *J. Exp. Med.* **133**, 169 (1971).
13. P. J. McCullagh, *ibid.* **132**, 916 (1970).
14. R. K. Gershon and K. Kondo, *Immunology* **18**, 723 (1970).
15. ———, *ibid.* **21**, 903 (1971).
16. K. U. Hartmann, *J. Exp. Med.* **132**, 1267 (1970).
17. W. D. Linscott and W. O. Weigle, *J. Immunol.* **95**, 546 (1965); W. E. Paul, G. W. Siskind, B. Benacerraf, *Immunology* **13**, 147 (1967). Our results differ in another fundamental aspect too; when cross-reacting antigens are used, the addition of the tolerated antigen prevents the breaking of tolerance. This difference does not necessarily mean that different mechanisms are operative in the two systems; but it is beyond the purpose of this report to discuss the possible reasons for this discrepancy.
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Loss of a Parathyroid Hormone–Sensitive Component of Phosphate Transport in X-Linked Hypophosphatemia

Abstract. *Mutant hemizygotes with X-linked hypophosphatemia lack a parathyroid hormone–sensitive component of inorganic phosphate transport in kidney; female heterozygotes retain a variable proportion of this type of transport. The residual mechanism for reabsorption in affected males allows inorganic phosphate efflux from the kidney to urine so that net "secretion" is sometimes observed; the latter is directly proportional to the serum concentration of inorganic phosphate. Calcium acts on the kidney tubule to enhance net reabsorption by this component of inorganic phosphate transport.*

The primary defect of X-linked hypophosphatemia has eluded clarification since the first descriptions of this form of vitamin D–resistant rickets (*1, 2*). Because of the impaired clinical responsiveness to vitamin D, and because of the discovery that intravenous calcium infusion could suppress the elevated renal clearance of inorganic phosphate (P_i) which accompanies hypophos-