Brief Definitive Report

THE ONTOGENY OF ANTIGEN-SPECIFIC T CELLS*

By ARTHUR M. SILVERSTEIN and SHRAGA SEGAL‡

(From The Wilmer Ophthalmological Institute, the School of Medicine, and the Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21205)

Evidence has been presented in the fetal lamb (1, 2), the opossum (3), and mouse (4) suggesting that the mammalian fetus attains immunologic competence to different antigens at different and discrete stages of its development. Thus, immunologic maturation appears as a well-ordered stepwise process, with some antigens able to elicit active immune responses very early in gestation and other antigens appearing unable to stimulate detectable responses until later in gestation or even during the neonatal period. It has always been tempting to identify the critical maturational event responsible for the sudden appearance of competence to a given antigen with the development of the antigen-specific receptors on immunocytes. However, Decker and co-workers (5, 6) have recently reported that antigen-specific B cells may develop in the fetus long before it is capable of forming antibody to that antigen. In the present report, we provide evidence that the appearance of functional, antigen-specific helper T cells in the fetal lamb also precedes the ability of the fetus to mount a specific antibody response.

Materials and Methods

The Experimental Protocol. Random-bred fetal lambs of known gestational age were immunized in utero, employing surgical techniques previously described (7). The technique of Rajewsky et al. (8), as extended by Katz et al. (9), was employed to assess the presence of helper T cells, using the following scheme. Six fetal lambs were given a priming injection of 1 mg of dinitrophenyl-keyhole limpet hemocyanin (DNP-KLH) intravenously in saline at 82 days gestation. 1 wk later, three of these animals received a supplementary injection of 100 μg of ovalbumin (Oval) in complete Freund's adjuvant distributed into intramuscular sites. At 105 days gestation, all of the animals received a booster injection of 1 mg of DNP-Oval intravenously in saline, and all animals were bled 7 days later at 112 days gestation.

Anti-DNP Antibody Assay. The technique employed for titration of anti-DNP antibodies was the bacteriophage plaque inhibition assay of Mäkelä (10) as applied by Segal et al. (11), using DNP coupled to T4 phage.

Results and Discussion

The data in Table I show that after priming of the fetus with DNP-KLH, only those animals which had received the ovalbumin supplement formed anti-DNP antibody when boosted with DNP on the second carrier, ovalbumin. Previous studies with this system had demonstrated that control animals immunized

* Supported in part by U.S. Public Health Service Research Grant HD-07935 from the National Institute of Child Health and Human Development, an unrestricted gift from Alcon Laboratories, Inc., and an Independent Order of Odd Fellows Research Professorship.

‡ Present address: Department of Cell Biology, The Weizmann Institute of Science, Rehovot, Israel.

TABLE I

DNP-T4 Phage Neutralization Assay for Anti-DNP Antibodies After Priming with DNP-KLH and Boosting With DNP-Oval With and Without an Intermediate Oval Supplement

Fetal lamb	Oval supplement	Dilution of serum for 50% neutralization
E26-1	Yes	1:10,000
E26-2	Yes	>1:10,000
E39-1	Yes	1:3,500
E3-2	No	<1:10
E25-1	No	<1:10
E39-2	No	<1:10

with DNP-KLH or DNP-ovalbumin alone by the intravenous route formed no anti-DNP antibodies. Since formation of anti-DNP on protein carriers is generally considered to be a T-cell-dependent process, it is evident that the ovalbumin-in-adjuvant supplement served in this instance to stimulate ovalbumin-specific T cells able to satisfy the helper function required by this system, as early as 112 days gestation in the fetal lamb.

Whereas hemocyanin is capable by itself of eliciting an active antibody response before 80-odd days gestation, we have found repeatedly that the fetal lamb is incapable of mounting an antiovalbumin response before 120 days gestation. These data would suggest, therefore, that ovalbumin-specific T cells may mature and function in the fetal lamb before full immunologic competence to this antigen is manifested. The present data, taken together with the report by Decker and Sercarz (6) that the appearance of ovalbumin-specific B cells in the fetal lamb also antecedes the attainment of competence to this antigen, raise again the question of the nature of the critical event that occurs at about 120 days to enable the fetal lamb to form antiovalbumin antibody. The precise timing of the maturational sequence of immunologic competence to different antigens at precise stages of development suggests that this is not a question of general control, but of specific recognition mechanisms, since other B and T-cell systems are present and working much earlier in gestation (e.g., bacteriophage ϕX 174 at less than 40 days gestation, ferritin at 56 days gestation, etc.—see ref. 2). These findings in the fetal lamb are consistent with the demonstrations (5, 12, 13) that both T and B cells may arise very early during ontogeny in other mammalian species.

It is, of course, possible that the critical maturational event for each antigen does not concern specific immunocytes, but rather reflects the sequential maturation of fetal ability to process appropriately the several antigens. Alternatively, since we have demonstrated the early presence of functional antigen-specific helper T cells, the critical event may operate at the level of the B cell. Thus, Mosier and Johnson (14) speculate that specific suppressor T cells may be present in excess during early development to inhibit normal B-cell function. However, we currently feel it more likely that an additional differentiative step may be required by the B cell, even after specific receptors develop. This event (the appearance of an *Ir*-gene product?) would then permit the B cell to translate antigen-receptor interactions into the signals required for the full-scale turn-on of genes necessary for the production of exportable antibody.

Summary

Although the fetal lamb is unable to form circulating antiovalbumin antibodies until about 120 days of gestation, ovalbumin-specific helper T cells can be stimulated to function at an earlier age. This would suggest that the critical event responsible for the precise sequential maturation of immunologic competence to different antigens at different developmental stages is not the first appearance of specific receptors on immunocytes. Alternative explanations of this phenomenon are discussed.

Received for publication 3 June 1975.

References

1. Silverstein, A. M., J. W. Uhr, K. L. Kraner, and R. J. Lukes. 1963. Fetal response to antigenic stimulus. II. Antibody production by the fetal lamb. *J. Exp. Med.* **117**:799.
2. Silverstein, A. M. 1973. An ontogenetic view of the generation of immunological diversity. *In* Phylogenic and Ontogenic Study of the Immune Response and its Contribution to the Immunological Theory. P. Liacopoulos and J. Panijel, editors. Institut National de la Santé et de la Récherche Médicale, Paris. 221.
3. Rowlands, D. T., Jr., D. Blakeslee, and E. Angala. 1974. Acquired immunity in opossum (Didelphis virginiana) embryos. *J. Immunol.* **112**:2148.
4. Sherwin, W. K., and D. T. Rowlands, Jr. 1974. Development of humoral immunity in lethally irradiated mice reconstituted with fetal liver. *J. Immunol.* **113**:1353.
5. Decker, J. M., J. Clarke, L. M. Bradley, A. Miller, and E. E. Sercarz. 1974. Presence of antigen-binding cells for five diverse antigens at the onset of lymphoid development: lack of evidence for somatic diversification during ontogeny. *J. Immunol.* **113**:1823.
6. Decker, J. M., and E. E. Sercarz. 1974. Early simultaneous appearance of antigen binding cells in the fetal sheep. *Nature (Lond.).* **252**:416.
7. Kraner, K. L., and C. J. Parshall, Jr. 1968. Experimental procedures and surgical techniques performed on intrauterine fetal animals. *In* Methods of Animal Experimentation. Vol. III. W. I. Gay, editor. Academic Press, Inc., New York. 211.
8. Rajewsky, K., V. Schirrmacher, S. Nase, and N. K. Jerne. 1969. The requirement of more than one antigenic determinant for immunogenicity. *J. Exp. Med.* **129**:1131.
9. Katz, D. H., W. E. Paul, E. A. Goidl, and B. Benacerraf. 1970. Carrier function in antihapten immune responses. I. Enhancement of primary and secondary antihapten antibody responses by carrier preimmunization. *J. Exp. Med.* **132**:261.
10. Mäkelä, O. 1966. Assay of anti-hapten antibody with the aid of hapten-coupled bacteriophage. *Immunology.* **10**:81.
11. Segal, S., A. Globerson, M. Feldman, J. Haimovich, and M. Sela. 1970. In vitro induction of a primary response to the dinitrophenyl determinant. *J. Exp. Med.* **131**:93.
12. Hayward, A. R., and J. F. Soothill. 1972. Reaction to antigen by human foetal thymus lymphocytes. *In* Ontogeny of Acquired Immunity. Ciba Foundation Symposium. Elsevier, New York.
13. Dwyer, J. M., and I. R. Mackay. 1972. The development of antigen-binding lymphocytes in foetal tissues. *Immunology.* **23**:870.
14. Mosier, D. E., and B. M. Johnson. 1975. Ontogeny of mouse lymphocyte function. II. Development of the ability to produce antibody is modulated by T lymphocytes. *J. Exp. Med.* **141**:216.