The Effect of Neonatal Thymectomy in the Induction of Experimental Autoimmune Thyroiditis in the Rat

Neonatal thymectomy in many species, including the rat, has been found to severely depress the development of those immunologic responses which have been termed cell-mediated. The ability of animals thymectomized at birth to reject foreign skin grafts is impaired[1,2], their lymphoid cells are incapable of eliciting a normal graft versus host response[3,4] and delayed hypersensitivity responses to such antigens as tuberculin and bovine serum albumin are depressed[2]. The effect of early thymectomy on the development of humoral immunity, however, has been found to vary considerably depending on the antigen and the species employed[5]. The purpose of the following experiments was to determine the effect of neonatal thymectomy in the rat on the ability to develop autoimmune thyroiditis, an experimentally induced disease characterized both by lymphoid cell infiltration of the thyroid and the production of antibodies to thyroglobulin.

Previous investigators have found that experimental autoimmune thyroiditis could be produced in the rat by the use of homologous or heterologous thyroid extracts or thyroglobulin emulsified in complete Freund's adjuvant[6] with or without *Bordetella pertussis*[7,8]. In the following experiments heterologous (bovine) thyroglobulin and Freund's complete adjuvant were used in combination with *B. pertussis* to produce thyroiditis in the rat.

Materials and methods. Neonatal thymectomy. Sprague-Dawley rats were thymectomized within 24 h of birth by a method described by ASANUMA et al.[9]. Rats were examined at autopsy for residual thymus tissue.

Production of thyroiditis. 6- to 8-week-old neonatally thymectomized or sham operated rats were injected in the hind footpads with 2 mg bovine thyroglobulin emulsified in Freund's complete adjuvant. A simultaneous but separate injection of 0.1 ml of *Bordetella pertussis* (200×10^9 organism per ml) was given s.c. in the upper surface of the hind legs. Control sham operated animals were injected in the same manner with bovine serum albumin. Animals were sacrificed by exsanguination at 17–18days following immunization.

Evaluation of thyroiditis. The thyroid gland of each animal was evaluated histologically by the following scale: 0, normal thyroid; + 1, varying numbers of small focal collections of lymphocytes; + 2, inflammatory foci of lymphocytes, some follicular disruption and colloid loss, approximately 25% of the gland affected, + 3, follicles more severely disrupted, some fibrosis, 25-40% of the gland affected.

Antibody titration. Anti-bovine thyroglobulin titers were determined by Ouchterlony percipitin reactions performed on microscope slides with 0.05 ml of a 4 mg/ml solution of bovine thyroglobulin or bovine serum albumin. Serial twofold dilutions of the antisera were prepared. The titer of precipitating antibody was determined as the last dilution giving a visible precipitate.

Results. 17 of 24 sham operated rats injected with bovine thyroglobulin, Freund's complete adjuvant and *Bordetella pertussis* developed thyroiditis which could be classified as + 2 (Table I). Sham operated control rats injected with bovine serum albumin, complete Freund's adjuvant and *B. pertussis* did not develop thyroiditis. Only 2 of the 9 animals showed lymphocytic foci on some sections of the thyroid. Neonatally thymectomized rats showed a greatly depressed ability to develop the same lymphoid cell infiltration of the thyroid that was characteristically seen in the sham operated animals (Table I). Animals that had been found to have thymic tissue remnants at autopsy showed thyroiditis equivalent to that of the sham operated animals (Table I).

The titers of precipitating antibody to bovine thyroglobulin were found to be the same in both the sham operated and neonatally thymectomized rats (Table II).

Discussion. Rats immunized with bovine thyroglobulin emulsified in Freund's complete adjuvant and given a simultaneous injection of *Bordetella pertussis* were found to develop moderately severe experimental autoimmune thyroiditis. This immunization method was found to be much more effective than the injection of bovine thyroglobulin and Freund's complete adjuvant alone[10]. The thyroiditis produced was characterized by the development of both lymphocyte infiltration of the thyroid and circulating antibodies to the bovine thyroblobulin, but it was found that only one of these parameters was affected by the removal of the thymus at birth. The neonatally thymectomized rat did not develop the lymphocyte infiltration characteristic of experimental autoimmune thyroiditis.

Previous studies[1,2], as well as the present investigation, seem to suggest that the cells involved in the induction of certain experimental autoimmune diseases are a class of thymus-dependent cell. In this research study and in those of others[2] even small remnants of thymus afforded the animal the ability to develop the experimentally induced disease.

Table 1. Depressed incidence of experimental autoimmune thyroiditis in neonatally thymectomized Sprague-Dawley rats as compared to sham-operated controls

Group	No. of animals	Histologic evaluation			
		0	+1	+2	+3
Sham-operated	24	—	4	17	3
Neonatally thymectomized	18	8	10	—	3
Incomplete thymectomy	5	—	1	4	—

Table II. Precipitating antibody titers to bovine thyroglobulin

		Log² antibody titer
Sham operated animals	24	2.54±0.66
Neonatally thymectomized	18	2.27±0.73

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Contrary to the depression observed in the production of the cellular infiltration of the thyroid, the neonatally thymectomized rats developed circulating titers of anti-bovine thyroglobulin which were equivalent to the titers seen in the sham operated animals. The production of circulating antibody to bovine thyroglobulin seems to be independent of the presence of thymus shortly after birth.

In these experiments it appears that both thymus-dependent and thymus-independent activities are involved in the response to bovine thyroglobulin. The thymus dependent mechanisms are mainly those that are involved in the direct cellular response in the thyroid, while the thymus-independent ones are primarily involved in the production of antibodies[12].

Résumé. Les rats Sprague-Dawley ont developpé une thyroïdite autoimmune caracterisée par l'infiltration de cellules lymphoïdes dans la thyroïde, qui fait suite à l'injection de thyroglobuline bovine, d'adjuvant complet de Freund et de *Bordetella pertussis*. Les rats nouveau-nés thymectomisés révélèrent une tendance nettement moindre à développer la thyroïdite.

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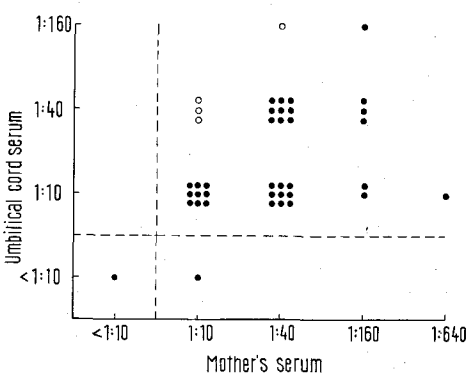
Simultaneous Determination of Antibody to Epstein-Barr Virus in Prenatal Mothers and New-Born Infants

Serological studies on the antibodies to EPSTEIN-BARR virus (EBV), originally found in cultures of Burkitt lymphoma[1], have suggested a close association of this agent to certain diseases such as Burkitt lymphoma[2], carcinoma of the posterior nasal space[3], infectious mononucleosis[4,5] and sarcoidosis[6]. Furthermore, anti-EBV antibody has been demonstrated in healthy persons living in different parts of the world[2,7-9]. The etiological role of EBV, however, in the above diseases and some of lymphomas and leukemias in man is not fully understood and the mode of its natural transmission remains largely uncertain. This communication reports the result of simultaneous determination of anti-EBV antibody titers in paired serums from a prenatal mother and her newborn infant.

Anti-EBV titers in test serums were determined by the method of other workers[2,6]. Cells of the P3HR-1 clone of the African Burkitt lymphoma-derived Jijoye cell line were used as a source of EBV antigen and indirect immunofluorescence was performed with serum diluted at 1:10, 1:40, 1:160, 1:640 and, if necessary, 1:2560. Mother's blood was withdrawn shortly before delivery and umbilical cord blood at the time of childbirth.

As shown in the Figure, in 38 of 40 pairs of mother and infant, both had detectable levels of anti-EBV antibody; the titers of mother's serum ranged from 1:10 to 1:640 and those of cord serum from 1:10 to 1:160. In 34 of the 38 pairs (closed circles) the mother's titers were equal to or exceeded the infant's titers, while in the other 4 (open circles) the infant's titers were higher than the mother's titers by a fourfold dilution. In one out of the 40 pairs, both mother and infant lacked anti-EBV antibody and in the last pair only the infant lacked antibody at a serum dilution of 1:10.

The present experiment has shown that the Japanese newborn infants had a 95% prevalence of anti-EBV antibody in the cord serum when tested at a dilution of 1:10 or higher, and there was a fairly good correlation between titers of mother and infant. Since anti-EBV antibody is restricted to IgG immunoglobulin, it would be readily transmitted from mother to infant through the placenta. Also, HINUMA et al.[9], in a study of Japanese population



Correlation of anti-EBV antibody titers in 40 pairs of mother and infant at the time of delivery.

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