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Ontogeny of Fc and Complement Receptors in Mouse Embryonic Tissues[1] (39010)

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It is now well-established that the monocyte–macrophage family of cells and B-lymphocytes possess separate membrane receptors for the Fc fragment of antibody combined with antigen (Fc receptors) and for antigen–antibody–complement complexes (C'3 receptors) (1–3). Although no functional role has as yet been ascribed unequivocally to these receptors, it has been suggested that the Fc receptor on B-cells may be involved in antibody-dependent lymphocyte-mediated cytotoxicity (4) and that the complement receptor serves either to concentrate antigen on the surface of B-cells (5) or acts as the recipient of the "second signal" in B-cell activation (6). The significance of these membrane binding sites in the function of macrophages is less well-defined.

In an effort to further understanding of the biological significance of Fc and C'3 receptors, studies were undertaken to outline their ontogeny in mouse embryonic tissues. Presented below are the results of observations made from the day after implantation (6 days) through birth (20 days). It was found that both receptors could be demonstrated on embryonic cells from the earliest time and that from the 8th day of gestation to birth their ontogeny paralleled that of the immune system.

Materials and methods. Fetal tissues were obtained from CBA/J pregnancies, and the age of the gestation was determined by considering the day the vaginal plug was observed as day zero. Embryonic material was carefully separated from the uterus in an effort to prevent or minimize contamination with maternal cells. The tissues were dissected as described previously (7), and identical organs from a single pregnancy were pooled, gently dissociated, washed and suspended in balanced salt solution at a concentration of approximately 10×10^6/ml.

Fc and C'3 receptors were demonstrated by testing the ability of cells to bind antibody–erythrocyte (EA) and antibody–erythrocyte–complement (EAC) complexes (2, 8). EA and EAC were made with 7S and 19S rabbit anti-SRBC sera, respectively (Cordis Laboratories, Miami, FL), and fresh mouse serum (1:10) was the source of complement for EAC.

The formation and enumeration of rosette-forming cells were performed by standard methods (8). Briefly, 0.1 ml of a 1% suspension of EA or EAC was added to 0.5 ml of cell suspension. The cells were gently centrifuged at 4°, carefully resuspended and examined in a hemocytometer under 400× magnification. A minimum of 2000 nucleated cells were scanned, and all positive cells (rosettes) had at least three erythrocytes firmly bound to their membrane. Because of the tendency for cells of embryonic origin to aggregate and thus assume a nonrandom distribution in the counting chamber, the data are presented as approximate values. However, each value presented represents the mean from at least five separate experiments. The specificity of EA rosette-formation was determined in all cases; heat-aggregated human IgG (50 μg/ml) inhibits EA but not EAC rosette-formation by competing for the Fc receptor site (9).

Results. Approximately 1% of the cells from 6- and 7-day gestations formed large rosettes with both EA and EAC (Tables I and II). The rosette-forming cells were very large (75–200 μm in diameter), extremely undifferentiated and did not appear to be of maternal origin, i.e., decidual cells.

EA and EAC rosette-forming cells were detected in the embryo on days 8 and 9 and in the yolk sac and placenta on days 8–10; thereafter, only a rare positive cell was found in extraembryonic tissues. In most instances these rosette-forming cells resembled those described above, but a small percentage of cells were smaller (12–20 μm in diameter)

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TABLE I. APPROXIMATE FREQUENCY OF EA ROSETTE-FORMING CELLS IN MOUSE EMBRYONIC TISSUES AT VARIOUS STAGES OF DEVELOPMENT.

	Rosettes/1000 cells Age of gestation (days)										
Tissue	6	7	8	9	10	11–12	13–14	15–16	17–18	19–20	Adult
Total gestation	12	14									
Embryo			4	10							
Yolk sac			7	5	3						
Placenta			8	6	3						
Liver					8	8	9	13	17	12	
Spleen										106	325

TABLE II. APPROXIMATE FREQUENCY OF EAC ROSETTE-FORMING CELLS IN MOUSE EMBRYONIC TISSUES AT VARIOUS STAGES OF DEVELOPMENT.

	Rosettes/1000 cells Age of gestation (days)										
Tissue	6	7	8	9	10	11–12	13–14	15–16	17–18	19–20	Adult
Total gestation	14	10									
Embryo			2	3							
Yolk sac			3	3	3						
Placenta			15	9	8						
Liver					3	4	11	12	19	51	
Spleen										175	362

and more differentiated, although it was not possible to establish their tissue type firmly.

On the 10th day of gestation just under 1% of fetal liver cells bound EA or EAC. The frequency of EA-binding cells remained relatively constant throughout pregnancy, but the incidence of cells able to bind EAC gradually rose to 5% at term. On the day before birth, 10% of spleen cells bound EA and 17%, EAC. Early in gestation the liver cells that bound EA and EAC were large and undifferentiated, but as pregnancy progressed up to 60% were small mononuclear cells with scanty cytoplasm and staining characteristics similar to those of small or medium-sized lymphocytes. Heat-aggregated IgG consistently inhibited EA but not EAC rosette-formation by all cell types at all stages of gestation.

When cells from embryonic brain, skin, extremities and subcutaneous tissue were tested it was found that approximately 10^{-4} or fewer were able to bind EA or EAC (data not shown). In all cases these positive cells were large and either undifferentiated or of the histiocytic type.

Discussion. Cells able to bind EA and EAC were first detected in embryonic material on the day after implantation. As differentiation continued, the yolk sac, placenta, fetal liver and spleen were found to contain the highest frequency of cells able to form rosettes. Thus, in a rough way there exists a parallel between the ontogeny of the immune system (7) and of cells that bear Fc and C'3 receptors.

On the other hand, the detection of these receptors on cells at least 2 days prior to the earliest known demonstration of lymphoid stem cells suggests that they may have functions outside as well as within the immune system. Although the rosette-forming cells found in tissues taken from 6- and 7-day gestations may have been macrophages or other members of the phagocytic series known to possess Fc and C'3 receptors, present evidence suggests that these cells differentiate in the same tissues and at the same time as does the immune system (9). Therefore, it is apparent that more work is needed to establish the identity of those early forms that bear Fc and C'3 receptors

and to determine whether these binding sites represent embryonic or phase specific membrane components that have been repressed or remain cryptic under normal conditions in all but the B-lymphocyte and the phagocytic cell. In addition, it would be of interest to know whether the blastocyst or earlier preimplantation forms have the ability to bind EA or EAC.

Conclusions. Mouse embryonic tissues were tested for their ability to form rosettes with EA and EAC. It was found that as early as the day after implantation (day 6) a small percentage ot cells was able to bind both. After the 8th day of gestation the development of rosette-forming cells paralleled that of the immune system. However, the detection of Fc and C'3 receptors on cells at least 2 days prior to the earliest known demonstration of lymphoid stem cells suggests that they may be expressed on cells other than those of the immune and phagocytic series.

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