Subcutaneous Enclaves in Mice as Sites for the Study of Cellular Responses In Vivo

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The enclave technique was devised to create a circumscribed lesion in connective tissue for observing cellular responses in vivo to bacteria, antigens, and biological materials such as toxins. This site is largely free of erythrocytes, tissue cells, and supporting structures, because the migrating leukocytes of the inflammatory reaction collect on the thin tissue membrane of the bacteria-air enclave. It differs from the connective tissue spread technique of Kolouch and others (1-3), and the granuloma pouch technique of Selye and Heuser (4) in the way it is established and in obtaining samples.

Preparation of the enclave. Mice were anesthetized by intraperitoneal injection of sodium pentobarbital (veterinary preparation) at 0.6 mg/g of body weight in a volume of 0.1 ml. The hair on the back was removed by electric clippers. The organisms for injection were of an 18-hr culture of *Neisseria meningitidis* (recently isolated from human spinal fluid; no. 791 of N. Vedros) grown on Mueller-Hinton agar slants under CO₂ at 37 C and suspended in Earle's balanced salt solution (Difco) or Isolyte (Baxter) multiple electrolyte solution to a viable count of 100 to 200 million per 0.5 ml. The standardized suspension in a volume of 0.5 ml was drawn into a 2-ml syringe fitted with a 26-gauge needle, 0.25 inch (0.63 cm) in length. One milliliter of air was also drawn into the syringe. The needle was inserted into a disinfected skin site, on the midline of the back of the mouse, to a depth between the subcutaneous layers of tissue and the musculature, but not to penetrate the muscles. Holding the mouse and syringe and needle at an appropriate angle upward permitted the introduction of the air first, then the suspension of organisms followed into the chamber created by the air bubble in the tissues. The enclave was a soft swelling, approximately 1 cm in height and 2 cm in diameter, which remained easily visible for about 1 week.

The mice were sacrificed at intervals and prepared for dissection by wetting the skin and fur

thoroughly with a Roccal solution of 1%. To prevent the undesirable entry of blood, cells of which invalidate the observations, a pair of sharp scissors was heated frequently in a flame before cutting the skin. A cut was made around the air enclave through the subcutaneous tissue, allowing a margin of skin to remain. The edges were lifted to reveal a thin membrane sac of connective tissue. This was carefully dissected away from the musculature of the animal's back, and the whole preparation of skin and intact air sac was laid upright on the autopsy tray (Fig. 1F). The bubble was cut, and the thin connective tissue was laid open to expose the lesion. Samples for cytograms were taken of the exudate by laying round cover slips (15-mm circles, no. 1 thickness) over the entire area of the lesion and allowing the fluid to flow onto the glass. These were gently removed by forceps, allowed to dry, then fixed in methyl alcohol and dried. Giemsa stain was used for cell study; after completion, the cover-slip circles were mounted with a drop of Permount (Fisher Scientific Co., Pittsburgh, Pa.) on the stained surface to seal the cover slip to a slide.

Results of differential counts of the cytograms from the enclaves are shown in Table 1. Controls, injections of only the suspending fluid and air, were included. Without particulate matter present, a smaller number of cells migrated to become a milder response of polymorphonuclear leukocytes, 11% compared with 71% at 24 hr in the mice injected with viable bacteria. At 48 and 72 hr, 95 to 100% of the cells were mononuclear, resembling those at zero-hour and 2 hr in test animals.

The enclave-cytogram technique has been shown in this example to be a means of observing the clearance of microorganisms from host tissue and of obtaining free cells for the cytological study of the host-parasite reaction. In the case of *N. meningitidis* in the mouse, the reaction was that of a highly nonsusceptible host which resisted

FIG. 1. Leukocytes from bacteria-air enclaves in subcutaneous tissue of mice after injection of *N. meningitidis* (Giemsa stain). (A) Response 6 hr after injection of *N. meningitidis*, polymorphonuclear cells predominating ($\times 250$). (B) Response at 6 hr ($\times 400$). Polymorphonuclear cells with phagocytized *N. meningitidis* and a few free-scattered meningococci. (C) Response at 48 hr ($\times 250$). Polymorphonuclear cells and macrophages. (D) Response at 48 hr ($\times 400$). Polymorphonuclear cells and macrophages. (E) Response at 96 hr ($\times 250$). Mononuclear cells with two transitional cells slightly above center of photograph. (F) Bacteria-air enclave dissected out on a section of skin from the back of the mouse to show the sac of thin connective tissue. Impression films of leukocytes in the illustrations were prepared on cover slips after such sacs had been laid open.

TABLE 1. *Cellular response in subcutaneous enclaves in mice to N. meningitidis*

Time of observation	Predominant cell of leukocyte response ^a			Number (approx.) of free <i>N. meningitidis</i> per oil immersion field
	Polymorpho-nuclear	Mononuclear	Effete	
Immediate	% 1	% 99	%	100-200 Culture: growth
2 hr		100 (3 Surface?)		100-150 Culture: growth
4 hr	61 (30)	1	38 (31)	50-100 Culture: growth
6 hr	66	<1	29	10-20 Culture: growth
8 hr	75 (34)	5 (9)	18	0-10 Culture: growth
23 hr	71 (2)	22 (None)	6 (None)	0 Culture: sterile
48 hr	55	36	8	0
72 hr	25	67	7	0
96 hr	7	82 3 Transitional	6	0
8 days	2	85 8 Transitional	5	0
14 days		100		0

^a Observed in count of 500 cells; five samples, except immediate and 2 hr, 100 cells each; lymphocytes, eosinophils 1 to 5% to complete 100% total, not shown. Figures in parentheses are phagocytic cells (per cent).

infection by 100 to 200-million organisms, and rapidly removed them within 24 hr. The model can be adapted for the study of in vivo reactions to other biological materials. Table 1 summarizes the cellular response, and Fig. 1A to E are examples of the cells in cytograms on cover-slip preparations. Fig. 1F is the bacterial-air enclave dissected from its site in the mouse.

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LITERATURE CITED

1. Hoessly, G.-F. 1954. Studies on immunization against plague. Schweiz. Z. Allgem. Pathol. Bakteriologie. 17(6):720-734.
2. Kolouch, F., Jr. 1939. The lymphocyte in acute inflammation. Am. J. Pathol. 15:413-428.
3. Page, A. R. 1964. Studies on the lymphocyte response to inflammation. Ann. N.Y. Acad. Sci. 116:950-963.
4. Selye, H., and G. Heuser. 1954. The induction of metastasizing transplantable fibrosarcomas with croton oil. Ann. Rep. Stress, 4th, p. 48-52. ACTA, Inc., Montreal.