

## Brief Definitive Report

### NONSPECIFIC SIGNALS FOR B-CELL LOCALIZATION AND ACTIVATION\*

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It is generally acknowledged that B lymphocytes rely primarily upon nonspecific signals (e.g., lymphokines and other chemotactic factors) to localize at the site of inflammatory reactions, or to home to lymphoid organs (1, 2). Most theories of antibody formation, however, require the obligatory participation of specific antigen with or without ancillary helper factors to account for the activation of these B cells to antibody formation (3-5). As pointed out by Coutinho and Möller (6), these theories all fail to explain the mechanism of action of certain plant lectins and other polyclonal B-cell activators which function independently of antigen. These authors indeed have suggested that the ultimate step in B-cell activation for immunoglobulin formation results from a single nonspecific mitogen-like signal, independent of the antigen-specific receptor site.

Apart from the extensive work on the more-or-less well defined polyclonal B-cell activators, there are few descriptions in the literature of antigen-independent B-cell activation. We have suggested that a nonspecific polyclonal response may accompany any specific primary or booster antibody response (7). Similarly, Jarrett and Bazin (8) postulated a polyclonal IgE response accompanying helminth infection, while Ferguson (9) reported the apparently antigen-independent activation of IgA-forming cells in heterotopic intestinal implants in mice. This report describes a study of the hormonal activation of virgin mammary autografts implanted into the anterior chamber of the rabbit eye. The data suggest that factors are released from the maturing gland which nonspecifically induce the immigration of predominantly IgA-committed B lymphocytes, and their antigen-independent activation to immunoglobulin synthesis.

#### Materials and Methods

*Animals.* New Zealand albino rabbits, all virgin does, weighing about 3 kg, were used. Examination failed to show any evidence of pre-existing eye disease.

*Anterior Chamber Transplantation.* Virgin, nonlactating mammary tissue was obtained in an aseptic manner through a small skin incision adjacent to a teat. The tissue was immediately placed into Hanks' balanced salt solution at 0°C, sliced into fragments about 2 mm on a side, and then autografted into the anterior chamber of the eye through a peripheral corneal incision (10). Other tissue fragments were prepared for microscopic observation.

*Hormonal Activation of Mammary Tissue.* After a period of at least 2 wk after anterior chamber

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implantation, the rabbits were given a single i.v. injection of 250 U of human chorionic gonadotropin (HCGT) (Organon Pharmaceutical Co., W. Orange, N. J.) in 0.25 ml. This is a modification of an earlier method (11). After 5–15 days, the animals were sacrificed, and the non-secretory orthotopic mammary gland tissue, as well as the implanted eyes, were removed for histological examination. Control animals received only i.v. saline injections.

*Antigen Injection.* In some animals containing mammary implants, a single intravitreal injection of ovalbumin (0.1 ml of a 1% solution in saline) was given into the same eye about 1 wk before sacrifice, to induce immunogenic uveitis (12). Several control animals received only intravitreal saline.

*Tissue Preparation and Fluorescent Antibody Technique.* Rabbits were sacrificed by an overdose of i.v. sodium pentobarbital. The eyes and orthotopic mammary tissue were removed and placed into 10% neutral-buffered formalin for 4 h and then transferred into 30% sucrose for 18 h. Serial sections were cut at 4  $\mu$ m in the cryostat, fixed in ethanol, and adjacent sections stained individually for rabbit  $\alpha$ ,  $\gamma$ , and  $\mu$  heavy chains by the direct method. At least two or more levels of each block were examined. Fluorescein and rhodamine conjugated goat anti- $\gamma$ , anti- $\alpha$ , and anti- $\mu$  rabbit heavy chains were obtained from Dr. John J. Cebra of the Biology Department, Johns Hopkins University, and from a commercial source (Cappel Laboratories, Inc., Downingtown, Pa.). Each reagent was tested on tissue sections of spleen, lymph node, and ileum to examine specificity and end-point dilution. In the antigen-inflamed eyes, areas of the uveal tract adjacent to and distant from the mammary implant were examined, as was the graft itself.

## Results

*Development of Orthotopic Mammary Gland after Hormonal Stimulation.* Rabbits were sacrificed before and at 5, 8, 10, and 15 days after receiving HCGT. The control, nonactivated virgin gland was quiet, with no acinar and little duct development noted. No Ig-containing cells could be found in the glandular tissue. However, at all time periods after hormonal activation, the ducts contained a whitish fluid. Acinar structures were present at 5 days, and were more numerous at later time periods. Plasma cells were present at all time periods, but were most prominent at day 8 and day 10, when 10–40 cells were seen per high power field. The overwhelming majority of cells stained for IgA, but an occasional IgG- or IgM-staining cell was seen (Table I).

*Behavior of Anterior Chamber Autografts after Hormonal Stimulation.* The mild clinical reaction usually subsided within 2 days after autotransplantation of mammary tissue to the anterior chamber. Control, nonactivated grafts were examined clinically and histologically in six eyes of six animals at various time periods between 2 wk and 6 mo after transplantation. All grafts were similar and, except for an increase in connective tissue, were identical to virgin orthotopic tissue.

Hormonal activation of anterior chamber autografts in four eyes of four animals led to changes comparable to those seen in orthotopic glands. Biomicroscopic examination showed a slight increase in overall size, with occasional cystic appearance on the graft surface. Histologically, the grafts showed a variable amount of fibrosis, but by day 8, acinar development, ductules, and plasma cells were present. The acini were dilated and filled with inspissated IgA- and secretory component-positive material (Fig. 1). Many IgA plasma cells were present throughout the gland interstitium, while few IgG or IgM cells were seen (Table I).

*Influence of Mammary Graft on Ig-Class Expression of Plasma Cells during Intraocular Inflammation.* 5 days after injecting 1 mg of ovalbumin into the vitreous of nontransplanted eyes, a mild anterior uveitis was present in all animals. The reaction was most intense by day 10–12, and then dissipated over the next 7–10 days. Numerous plasma

TABLE I  
*Differential Counts of Ig-Forming Cells in Inflamed Eyes Containing Mammary Gland Autografts*

Hormonal activation	Tissue	Percent of total Ig cells*		
		$\alpha$	$\gamma$	$\mu$
+	Mammary gland	88	5	7
+	Adjacent uvea	25	63	12
+	Distant uvea	12	80	8
-	Mammary gland	Too few cells to count		
-	Adjacent uvea	11	81	8
-	Distant uvea	9	84	7
+	Mammary gland in Uninflamed eye	90	7	3
-	Mammary gland in Uninflamed eye	Too few cells to count		

\* Total Ig cells are the sum of  $\alpha$  +  $\gamma$  +  $\mu$ .  $\epsilon$  and  $\delta$  cells were not counted.

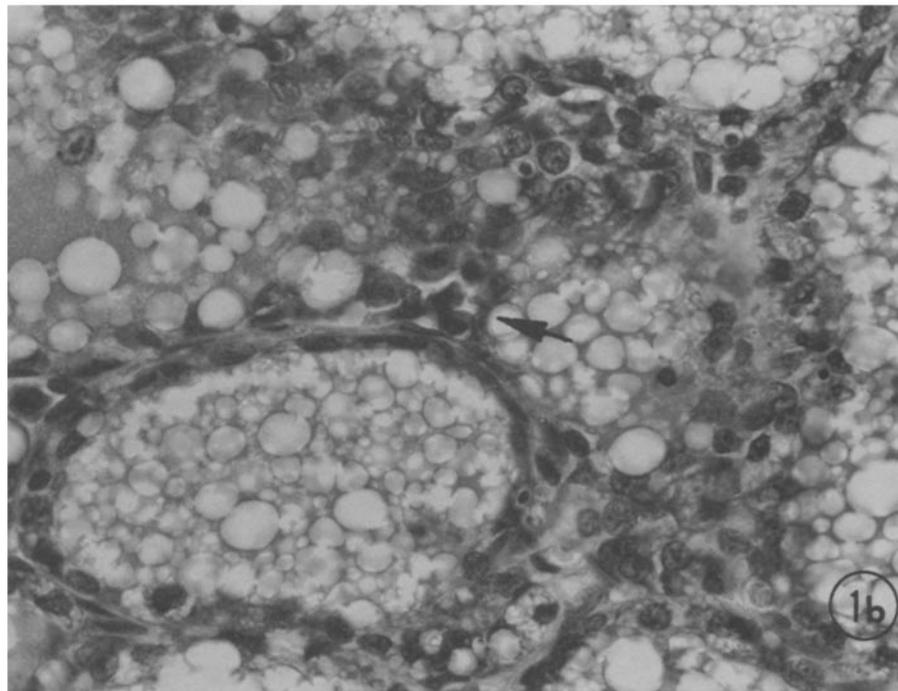
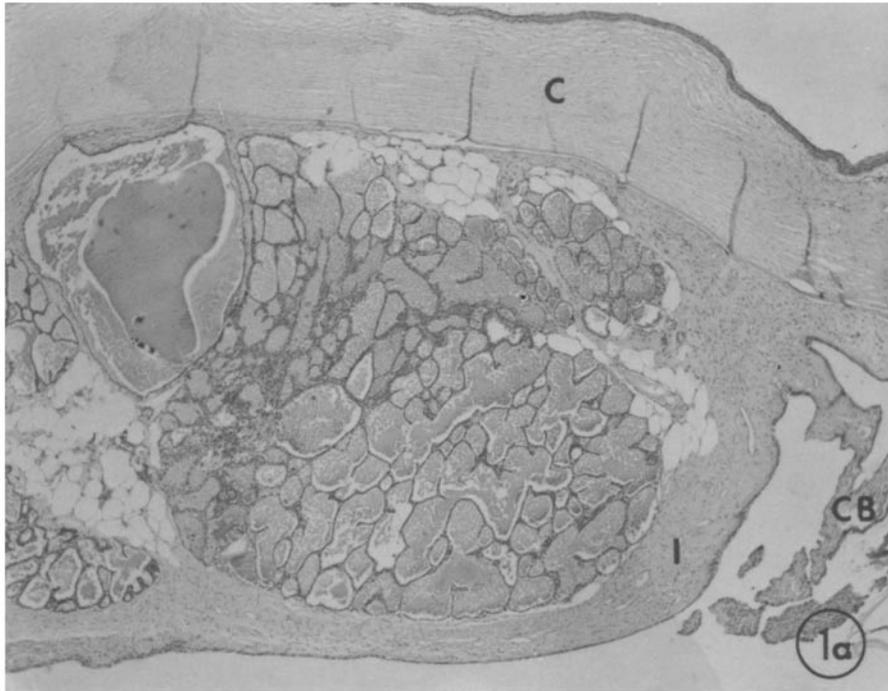
cells were evident in the intense inflammatory reaction at 10–12 days after antigen injection, the majority staining for IgG, with only 7–10% of cells in the iris and ciliary body stained for IgA.

Six eyes of six animals containing nonactivated anterior chamber grafts received intravitreal antigen. Histological examination at 10–12 days revealed numerous plasma cells within the intraocular inflammatory reaction, but few plasma cells were seen within the mammary graft. Plasma cells within the iris and ciliary body showed little change in the percentage of IgA cells adjacent to the inactive graft (Table I).

Animals with hormonally activated anterior chamber grafts given intravitreal antigen showed a significant increase in IgA plasma cells in the iris and ciliary body adjacent to the graft, with no increase in IgA cells at sites distant from the graft (Table I). There was also a slight increase in IgM cells in the adjacent uvea as compared to the distant uvea of the same eye.

### Discussion

The factors responsible for the specialized trafficking and homing of certain lymphocyte subclasses are not well understood, although they are generally suspected of being independent of the action of specific antigen (1, 2). In contrast, the activation of B cells to the specific production of antibody is believed by most investigators to require the specific binding of antigen upon cell membrane receptors. The antigen-receptor signal is thought to function alone in the case of T-independent antigens (6), or in concert with other helper factors in the case of T-dependent antigens (5). In either event, only the antigen-specific cells are thought to be activated, and little attention has been paid to the possibility that other unrelated B cells may be polyclonally activated during this process. Only a few suggestions that this phenomenon may exist appear in the literature (7–9). However, the function and significance of plant lectins and other B-cell polyclonal activators has been considered in detail by Coutinho and Möller (6), who point out that most theories of antibody formation fail to account for polyclonal B-cell activation. These authors have suggested that the final signal for B-cell activation may be due to a nonspecific polyclonal factor, although they do not consider in detail the origin or mechanisms of release of this factor(s).



**FIG. 1.** Implant of hormonally activated mammary gland in the anterior chamber of the rabbit eye. (a) Low power view showing the graft filling the anterior chamber, and its relationship to the cornea (C), iris (I), and ciliary body (CB). (Hematoxylin and eosin,  $\times 30$ ). (b) Higher power showing a collection of plasma cells between swollen acini containing fat droplets and milk. (Hematoxylin and eosin,  $\times 600$ ).

We earlier suggested that every interaction between B cells and their specific antigens may be accompanied by the formation of such polyclonal B-cell activators, based upon the demonstration of a high proportion of nonspecific immunoglobulins formed during the course of both primary and booster antibody reactions against specific antigen (7). In the present study, we have extended these observations in a demonstration that even in the antigen-free environment afforded by the anterior chamber of the rabbit eye, the hormonal activation of virgin mammary tissue results in the accumulation of B cells and their activation to Ig formation, concomitant with the development of milk production. This would suggest that even a hormonally activated nonlymphoid organ may release factors that cause the localization and polyclonal activation of B cells. Being an integral part of the secretory immunological system, it is to be expected that this phenomenon would involve primarily IgA-forming cells, whereas our earlier report of polyclonal B-cell activation accompanying specific antibody formation within the uveal tract involved principally IgG and IgM cells. Preliminary data suggest that these nonspecific factors are probably diffusible, since increasing numbers of IgA-forming cells were found in the uveal tract adjacent to the mammary gland implant as compared with control sites distant from the gland, or in nonimplanted eyes.

The phenomenon of release of nonspecific lymphocyte-attracting and -activating factors by various tissues may be a general one. It is well known that at very specific stages of ontogeny, the regional lymph node, the spleen, the gut-associated lymphoid tissues, and various secretory glands each becomes populated by a variety of lymphocyte subclasses which are activated to fulfill at least some of their functions, all in the apparent absence of antigen. The phenomenon seems especially appropriate in the case of the mammary gland, because a mechanism for the nonspecific accumulation and polyclonal activation of maternal B cells at the onset of lactation would efficiently furnish to the suckling young protective antibodies representing a fair sample of the immunological history of the mother.

We do not mean to suggest here that the nonspecific polyclonal activator signal is the only one that can activate a B cell, as proposed by Coutinho and Möller, although the data do support their notion that B cells must have receptors for such polyclonal activators (as well as for other lymphocyte-localizing factors). Rather, it is our present belief, based upon still-inadequate data, that the specific antigen-receptor signal and the nonspecific polyclonal signal may each alone be sufficient for the triggering of B cells.

### Summary

Virgin, inactive mammary gland autografted to the anterior chamber of the rabbit eye remains free of lymphoid cells. Activation of the ectopic gland by systemic injection of chorionic gonadotropin results in maturation of the gland and milk production, accompanied by the immigration of lymphocytes and their activation to Ig formation, predominantly of the IgA class. In the presence of antigen-induced intraocular inflammation, the activated gland is able to influence the Ig class of B cells in the neighboring ocular tissues. These data suggest that even nonlymphoid tissues may elaborate lymphocyte-homing and polyclonal B-cell activating factors which function independently of specific antigen.

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