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EDITORIALS

Cellular Aspects on the Phylogeny of Immunity

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One way of studying the development of an organ system is to start the study in a phylum in which the actual organ system has reached the highest degree of organization or complexity, and then turn to less organized or less complex organs in other phyla. It is wise to keep in mind that there are no fossil records of most organ systems. This is certainly true of the lymphatic organs. The fishes deviated from our common ancestors about 300 million years ago, and modern fishes thus had a long time to develop fancy lymphatic organs if the selection pressure was such as to require this. We can thus expect to find a variety of "solutions" to the same basic problem. A study of the various "solutions" may also give some ideas towards what the "problem" was, in other words: Why lymphatic organs developed at all.

First Level and Second Level Lymphoid Organs

The last ten years of immunological research have given us good reason to divide the lymphoid organs into first level and second level. If a first level lymphoid organ is removed early enough in ontogeny development of certain parts of the second level lymphoid organs is reduced in the operated animal. First level lymphoid organs defined so far are the thymus and bursa Fabricii of chickens. The work of Cooper, Peterson and Good (1) has indicated that there is a dissociation of the immunological function in the chicken. The bursa of Fabricius controls the development of immunoglobulin production, and the thymus is largely responsible for the ontogeny of cellular immunity.

Thymus and bursa Fabricii are both lymphoepithelial organs in the sense that they represent an intimate relationship between epithelial or epithelial-derived cells and lymphocytes. Lymphocytes in both organs are characterized by rapid proliferation. It has been shown (2, 3) that thymus and bursal lymphocytes are at least partly derived from blood borne progenitor cells, which enter the epithelial primordia of these organs during histogenesis.

The mechanism behind the influence of thymus and bursa Fabricii on the ontogeny of the rest of the lymphoid organs, the second level lymphoid organs, is not known. There is good evidence for a cellular theory according to which an export of lymphocytes from the first level organs explains the effect. There is also evidence for a hormonal theory at least in the case of thymus which does not necessarily exclude the cellular one (4, 5). First level lymphoid organs may offer a microenvironment during normal ontogeny necessary for the maturation of lymphocytes, and, therefore, also necessary for the maturation of lymphoid organs. This-maturation effect or instructor function seems to be of importance for the whole life of the individual organism.

A lymphocyte is thought of as immunologically non-competent until it has become competent within the first level lymphoid organ or after a stay in such an organ. The immunologically competent lymphocytes then become committed to a certain type of antibody formation and clones of committed lymphocytes are formed within the second level lymphoid organs.

In a "pure" first level lymphoid organ there is no, or practically no, antibody formation. It is important to keep in mind that a lymphoid organ may function as both a first level and a second level lymphoid organ, in which a lively antibody production takes place.

An interaction between thymus lymphocytes and bone marrow lymphocytes in the hemolysin producing system has been demonstrated (6, 7, 8). As antibodies are mainly formed in the second level lymphoid organs, such as lymph nodes and spleen in mammals, cell migration from the thymus to the second level lymphoid organs demonstrated in many laboratories is congruent with this thymus-bone marrow relationship in antibody production. Very little is known about the bone marrow cells taking part in this cell to cell interaction. We do not know, for instance, if they are bursa dependent, in other words, if they have to become instructed within the bursa Fabricii or its equivalent in the same way as bone marrow lymphocytes are thought of as being instructed within the thymus.

All animals phylogenetically more recent than the cyclostomes have a thymus and are capable of performing thymus dependent functions, i. e. cellular immunity (9). All animals phylogenetically distal to the cyclostomes display a bursa dependent function, i.e. immunoglobulin production, but only birds have a bursa Fabricii (9). There is growing evidence that the *Peyer's* patch type of tissue in rabbits, another lymphoepithelial organ, has a bursa function (10, 11).

Theoretical Considerations about the Very Early Phylogeny of Immunity (Fig. 1)

The first places where external antigens are met with by the primitive organism are the outer and inner surfaces of the body. Internal antigens, or "not self", originate from mutations, which most likely appear in rapidly proliferating tissues. The gut epithelium and the epidermis do belong to the most rapidly proliferating tissues of such animals. Internal antigens thus most likely appear at the same site as external, at the inner and outer surfaces of the organism. This leads to the suggestion that the first antigen reactive cells are epithelial cells, in epidermis and in the gut, and that the first primitive antibodies

are formed by epithelial cells. Primitive antibodies produced by epithelial cells may very well precede antibodies produced by lymphoid cells in phylogeny. The lymphoid cells may have come to help at a higher level of organization, when epithelial cells had to become free to specialize in other directions. This can be looked upon as a parallel to the development of red blood corpuscles (from the same stem cells?) coming to help the growing organism in oxygen transportation.

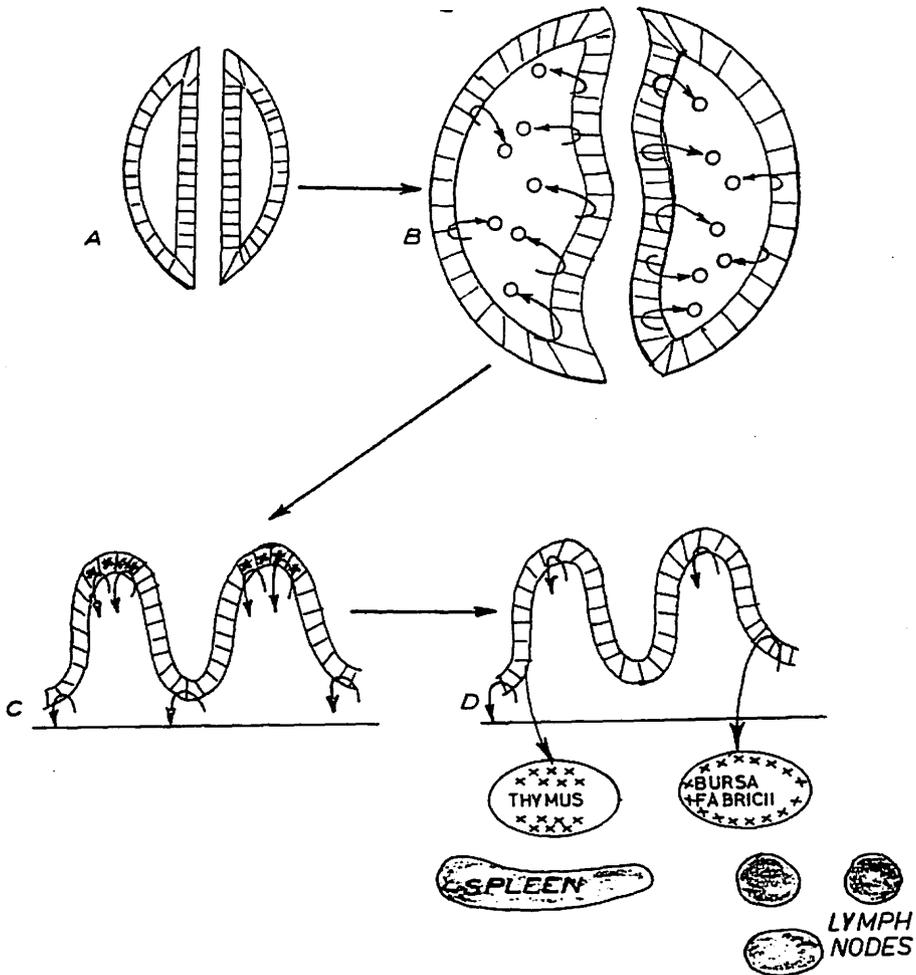


Fig. 1 Development of the immune system. A. The primitive organism meets antigen, both external antigens and "not self", on the surface. Epithelial cells react to antigen. B. Lymphoid cells come to help. First they may react to antigen within the epithelium. Later they may become instructed within the epithelium, move away and perform their immune function somewhere else. C. The instructor function becomes concentrated to certain areas. D. Finally some instructor organs move from the surface. At this stage organized second level lymphoid tissue develops.

To start with, the lymphoid cells might have done their job within the epithelium itself. Later in development they had to do it somewhere else after having received some kind of instruction from the epithelial cells. In the beginning this hypothetical instructor function of epithelial cells may have been exerted by all surface cells. When this function became concentrated to certain areas of the epithelium, there could certainly be different instructions going on in different locations, depending on the antigen which initiated the instruction at the particular site. In birds there are at least two such locations, the thymus and the bursa Fabricii, offering at least two types of hypothetical instruction. In mammals there are many types of lymphoepithelial locations known. In one of them, the thymus, instruction of lymphocytes has been anticipated (4). There may be other types of instructions going on in the tonsils, in the Peyer's patches, in the epithelium of the gut, in epidermis. This theory explains why the lymphocytes made contact with the epithelium in the first place, and why the epithelium is leading during the development of the lymphoid system. It means, that the phylogeny of immunity starts among the invertebrates.

Diffuse and Circumscribed Lymphoepithelial Relationships of Vertebrates

The new concept of first level and second level lymphoid organs, and the fact that all the first level lymphoid organs defined so far are lymphoepithelial, gives a new meaning to the old classification of the lymphoepithelial organs as a particular entity among the lymphoid organs. It also justifies a new close look at all types of lymphoepithelial relationships in a phylogenetic and ontogenetic perspective. This has recently been done.

There are lymphocytes in nearly all epithelia of the vertebrates. Special attention has recently been devoted to the gut epithelium and to epidermis (12, 13, 14). The data collected so far on lymphocytes within these epithelia are difficult to interpret. It is, however, safe to say that they are well in line with the idea that a selected number of young lymphocytes of the blood enter the epithelium, and after a relatively short stay (a matter of days) reenter the circulation. The lymphocytes reaching the epithelium to a large extent may be non-competent lymphoid cells, which become competent during or shortly after a stay in the epithelium.

It was postulated in the previous paragraph that the lymphoid cells might have started by doing their job within the epithelium itself. This justifies mentioning that the lymphocytes within the gut epithelium of certain amphibians are pyroninophilic (13), which may indicate that these cells are producing antibodies.

A number of "new" lymphocyte collections in close relationship to the gut epithelium have been described in fishes, and reptiles (15), (see Fig. 2). A reexamination of the lymphoepithelial organs of homo sapiens, based on studies of the literature and on new observations in newborn children, has been made by *Fichtelius* et al. (16). Again "new" lymphocyte collections in close relationship to epithelium were described, most of which seem to be present in the human organism already before birth (see Fig. 3). It is less probable that these accumulations are all consequences of inflammation at individual

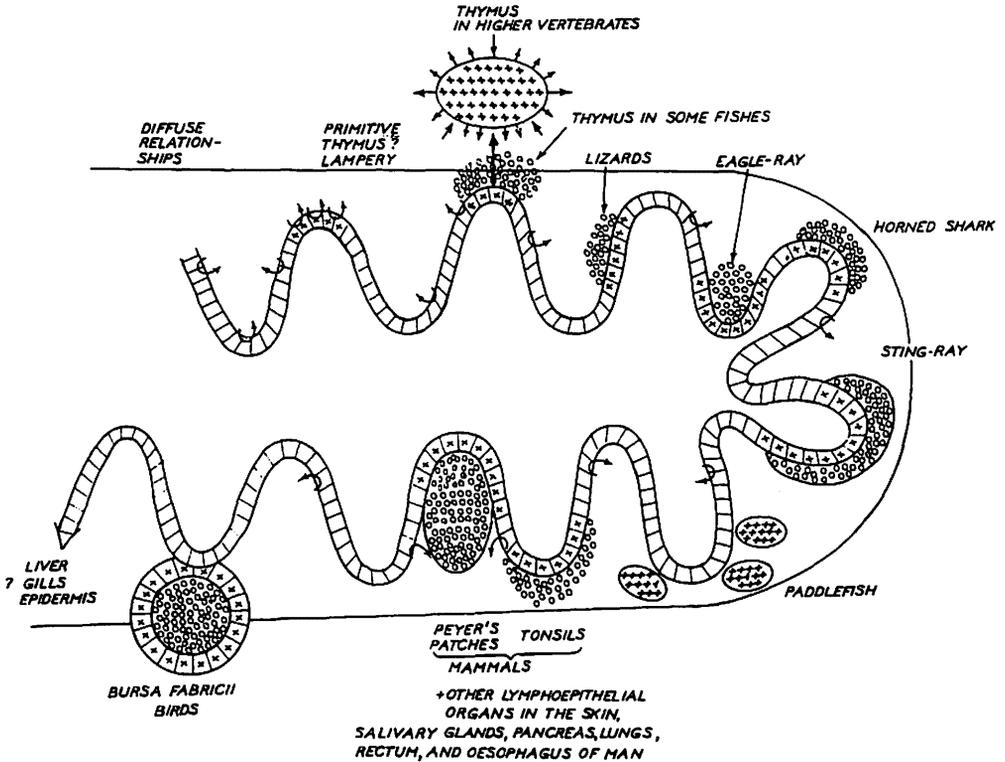


Fig. 2 Different types of gut-associated lymphoepithelial tissue of vertebrates in a stylistic gut section. All these formations have one thing in common – a close spatial relationship between epithelium and lymphoid cells. They may all be examples of first level lymphoid organs.

levels. On the other hand it is reasonable to assume that we are dealing with small lymphoepithelial organs of different shape and size. They may all be first level lymphoid organs, partial equivalents to the bursa Fabricii of birds.

Current Views

It is of immediate interest to see in which way the above mentioned concept of the early phylogeny of immunity is similar or different to current views.

Burnet and Fenner (17) were the first to emphasize the probable importance of recognition to the immune response. They pointed out the apparent need for recognition of "self" and "not self" at a molecular level in holozoic organisms from a nutritional point of view. A mechanism that can distinguish foreign protein molecules from among a multitude of self-synthesized protein molecules enables a typical animal nutrition. Of course the evolution of the digestive system to a tract, the lumen of which is essentially outside the organism, has decreased the need for recognition in nutrition. However, the possession of a highly developed digestive tract typical of vertebrates is not the general rule among the animals. Many of the animal phyla are characterized by digestive tracts

Lympho-epithelial organs of homo sapiens.

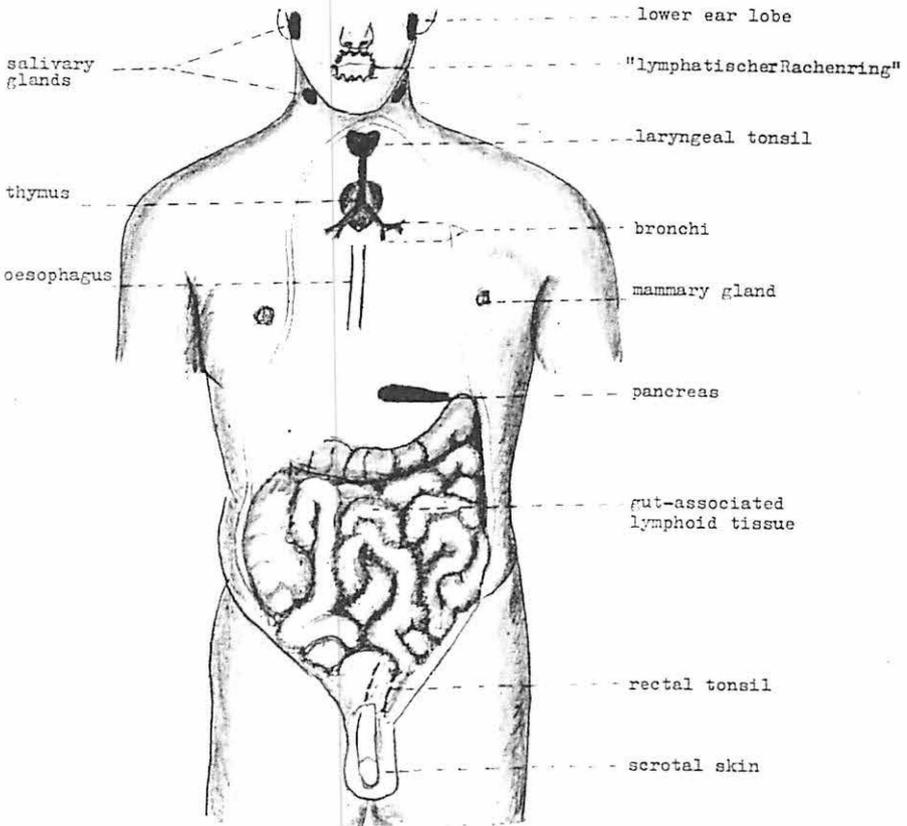


Fig. 3 The location of old and "new" lymphoepithelial organs of homo sapiens. Some of them have up to now been described as pathological lesions.

in which lining cells along with other phagocytic elements accomplish much of digestion as an intracellular process, and thus need some kind of recognition system. So far the concept of *Burnet* and *Fenner* is in line with the theory put forward in this article.

Hildeman (18), discussing a paper by *Good* and *Papermaster* (19), claims that specific immunologic competence demonstrable in cellular and/or humoral responses probably exists in eumetazoan invertebrates. He writes: "Phagocytic mechanisms are, of course, highly developed in invertebrates as well as vertebrates. Numerous and diverse leucocytic cell types with distinctive cytoplasmic endowments are known in eumetazoan invertebrates, but their specialized functions are almost entirely unknown. That intracellular digestion of potential antigens should lead to persistent specific responses only among vertebrates beginning at the level of elasmobranchs seems anomalous". There is

no longer any doubt that invertebrates have specific immunological memory (20, 21, 22, 23, 24, 25, 26, 27) and it is certainly easy to subscribe to *Hildeman's* views today (28).

There is, however, one distinct difference between *Hildeman's* approach and mine. *Hildeman* thinks of the numerous and diverse leucocytic cell types in the invertebrates

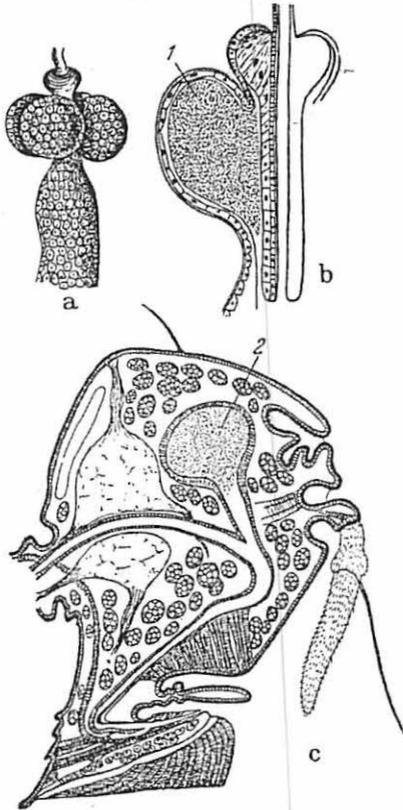


Fig. 4 a) and b) The four blind sacs of the larva of the fly *Dacus oleae* filled with bacteria.

Fig. 4 c) Section through the head of a mature female. 1. Symbionts. 2. The single sac of oesophagus with symbionts. According to Petri.

and their unknown functions. In this article the primary importance of epithelial cells in immune response is emphasized.

— *Burnet* (29) has recently discussed the evolution of the immune response from a cellular point of view. One of his basic assumptions is that adaptive immunity is characteristic of vertebrates only. He thinks that the absence of adaptive immunity in insects and other invertebrates depends on the failure of specific stimulation of a reactive hemocyte to provoke its multiplication. By hypothesis the response of the wandering cell to specific contact is limited to damage and conversion to an encapsulating fibroblast-like cell. The evolutionary step towards an adaptive response was the acquisition of the potentiality of a specifically patterned mobile cell to respond to at least a proportion of specific contacts by proliferation to produce a descendant clone of the same specificity.

Again the discussion is circling around the primitive mobile cells of invertebrates and around their lack of lymphocytes. The role of epithelial cells is not taken into account.

The concept of *Good et al.* (30, 31, 32) of the local antibody production in the gut as a late event in phylogeny is contrary to the theory expressed in this article. They think it is striking that, despite the presumed maximal antigenic exposure occurring via the gastrointestinal tract in the lower vertebrates, the plasma cell system appeared latest at this location (30). This observation is taken as

support for the theory expressed by *Thomas* (33) that the pressure for development of the lymphoid and plasma cell systems may, at their inception, have been primarily intrinsic rather than extrinsic, i. e. a means of control of aberrant cell development and not primarily a defense against microorganisms.

According to the theory advocated in this article it might very well be so that primitive antibodies were formed by the epithelial cells of the gastrointestinal tract before there even were any animals with lymphocytes.

*A Comparison between the Lymphoepithelial Organs of Vertebrates
and the Symbiont Organs of Invertebrates**

Among the invertebrates lacking lymphatic organs and lymphocytes there is a variety of symbiont organs – special organs harbouring more or less pure cultures of different microorganisms with which the hosts are living in symbioses (35). These organs can look practically any way and they can be located practically everywhere. In this respect they resemble the lymphoepithelial organs of the vertebrates. They can be bursalike, and the spatial relationship between the host and the symbionts can be diffuse (Fig. 4, 5). They can all be described as lymphoepithelial organs devoid of lymphocytes.

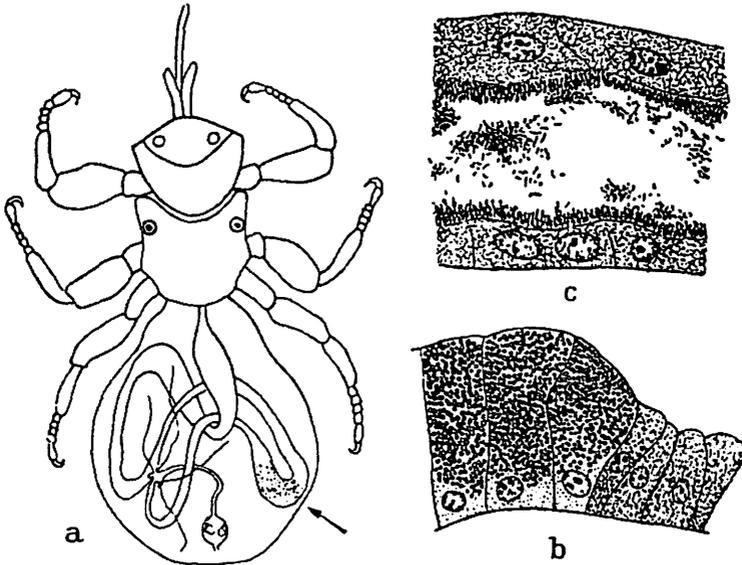


Fig. 5 a) A louse – *Melophagus ovinus* – with an arrow at the part of the gut which is contaminated with symbionts. According to Zacharias. b) The border between the sterile and contaminated part of the gut of another louse – *Lynchia maura*. According to Aschner. c) Symbionts in the gut lumen of a third louse – *Hippobosca equina*. According to Aschner.

The parallel morphology between the lymphoepithelial organs of vertebrates and the symbiont organs of invertebrates gives, of course, rise to many questions that cannot be answered at the moment. Did the lymphoepithelial organs evolve from symbiont organs? Was symbiosis there before immunity? Are some of the lymphoepithelial organs of modern vertebrates symbiont organs? Do the symbiont organs of invertebrates have some kind of immune function?

It is of particular interest to reexamine the only symbiont organs of vertebrates clearly defined so far, the light organs of certain teleosts (35, Fig. 6). A check of the

* I am very grateful to Dr. *Bengt Gustavson*, Karolinska Institutet, Stockholm, who described these organs to me, and gave me access to his superior knowledge within this field.

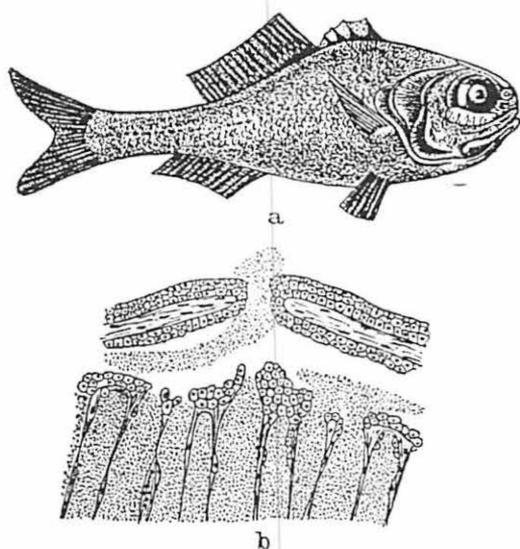


Fig. 6 A teleost - *Anomalops kataptron*. a) Total view. The light organ can be seen under the eye. b) Opening of the light organ: its slits are filled with bacteria. According to Steche.

(Fig. 4-6 are taken from: P. Buchner. *Tiere als Mikrobenzüchter*. Springer-Verlag, Heidelberg 1960)

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available literature reveals that lymphocytes or collections of lymphocytes are certainly not a prominent feature of these organs.

The role of lymphocytes in the evolution of lymphoid organs is of course very important. It might be, however, that the role of epithelium has been overlooked, since the epithelium seems to be leading in ontogeny and might be leading in phylogeny. So far at least one thing can be learned by immunologists from the mere existence of symbiotic organs in invertebrates; interaction between microorganisms and epithelium can result in the specialization of the epithelium and even in the formation of rather complex organs without the participation of lymphocytes.

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An Experiment in the Teaching of Lymphology to Medical Students

Internal Medicine A-108: Lymphovascular System

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Lymphology 1 (1970), 59—61

In the fall of 1967, the five charter members of the Greater St. Louis Lymph Club (*P. Ruben Koehler* and *E. James Potchen*, Department of Radiology, *Marlys Hearst Witte*, Department of Medicine, *Charles L. Witte* and *William R. Cole*, Department of Surgery, Washington University School of Medicine), local chapter of the International Society of Lymphology, met in the downstairs room of a local pub. Out of this meeting arose the idea of an elective course in Lymphology as part of the new fourth-year medical curriculum at the School of Medicine. The course was to provide a multidisciplinary approach to the newly born field of experimental and clinical lymphology and was to consist of seminars, laboratory experiments, and clinical demonstrations. Nine seniors elected the 12-week course (one 2-3 hour session per week), which was taught twice during the 1968-1969 academic year.