

INTERACTION BETWEEN LYMPH NODES DURING IMMUNIZATION

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Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 54, No. 9,

pp. 81-84, September, 1962

Original article submitted October 12, 1961

By now a large amount of material on the antibody formation of lymph nodes has accumulated. It has been shown that under certain conditions, during the formation of the antibodies not only are the regional lymph nodes involved but so also to various degrees is the whole of the lymphoid system [1-4, 7-9, 15, 21, 22]. The reasons for this systemic reaction are largely unknown. Previously [1, 7, 8] we showed that the systemic reorganization of the immune response of remote lymphatic nodes cannot be caused exclusively by the action of the circulating antigen. We suggested that for the complete response of the remote nodes to develop, they had to be stimulated in a particular way from the regional lymph node. To test this hypothesis we had to determine what would be the influence on the immune response of the other lymphatic nodes and of the whole organism, caused by removal at operation of the regional lymph node.*

METHOD AND RESULTS

Injection into the right rectus femoris of 0.5 ml of tetanus antitoxin (series 336, Gamalei Institute of Experimental Medicine) was used to immunize 33 male chinchilla rabbits weighing from 1.9 to 2.6 kg. After three days, the regional iliac lymph nodes were removed from ten rabbits under ether anesthesia. The nodes were homogenized in physiological saline or in distilled water. The homogenate of the nodes of seven rabbits was tested to determine the amount of anatoxin and antitoxin present, and a homogenate of the nodes of three rabbits was injected into the same animals from which the nodes had been removed. The remaining animals served as controls: from them either the contralateral lymph nodes were removed at the same time (7 rabbits), or the trunk was cut and the nodes left in place (in 5 rabbits), and 11 rabbits were left unoperated. After two months, all the rabbits received an injection of the same dose of antigen into the skin of the left ear. The animals were killed three days after revaccination, the parotid and cervical lymph glands were removed, homogenized in physiological saline, and the amount of antitoxin in the homogenates was determined by bio-assay on mice (see table).

As can be seen from the table, in rabbits from which the regional lymphatic nodes have been removed, the parotid and cervical glands reacted to the injection of the antigen much less strongly than they did in the controls. The normal immune response could not be restored by injecting back a homogenate of the lymphatic glands previously removed, a result which demonstrated the absence of antigen in these glands. The absence of measurable amounts of antigen in the removed lymphatic glands (less than 1% of the amount injected) was observed also in titration of homogenates by a modified Becher-Kraus method [5]. Previous investigations [6] have shown that at the site of injection of the antigen (the muscle), after three days, less than 5% of the injected antitoxin is present. In the removed lymph glands, not only antigen, but also antitoxin was absent.

Therefore, removal of the regional lymph glands three days after immunization had no appreciable effect on the resorption of antigen by the other lymph glands; also, the fact that the immune response of the latter was considerably reduced as a result of the operation constitutes a definite proof of the interrelationship between the lymph glands, an interrelationship of a particular kind, which is not to be attributed to regulation of resorption of the antigen.

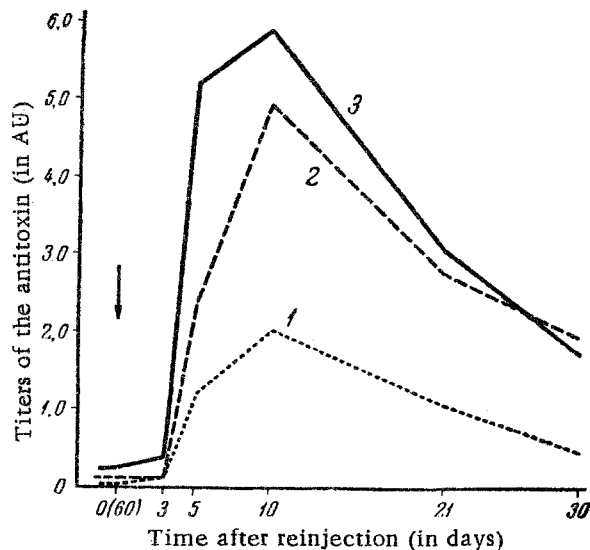
However, it is relevant to consider what part is played in the general immune response by the interaction between the lymphatic nodes. To find out, we immunized 24 rabbits by the injection of 0.5 ml of tetanus anatoxin into

*Influence of removal of the regional lymph nodes during the primary immune response has been studied previously [8,22].

Influence of the Extirpation of the Regional Lymphatic Nodes on the Reorganization of the Immune Response of the Remote Nodes

Experiment	Operation	Number of rabbits	Concentration of antitoxin (in AU per g) in the parotid and cervical nodes, after revaccination
First	Removal of the regional iliac glands after the first immunization	7	0.11 ± 0.07
	Removal of the contralateral nodes	7	1.62 ± 0.56
	No operation	11	0.90 ± 0.10
Second	Removal of the regional nodes, and injection of their homogenates	3	< 0.02
	Incision into skin and muscle	5	1.00 ± 0.33

the right rectus femoris. The animals were then divided into three groups, each containing eight rabbits. In the first group, after three days, the right iliac lymphatic glands were removed under ether anesthesia; a mock operation, consisting of an incision into the skin and muscle, was performed on the remaining two groups. Seven weeks after immunization, the corresponding lymph glands were removed from the rabbits of the second group; mock operations were performed on the first and third groups. Ten days after the second operation, all the animals received an injection of 0.5 ml of anatoxin into the skin of the ear. We then measured the amount of antitoxin in the blood serum at different times after the second injection.



Titers of antitoxin in the blood serum of animals after removal of the regional lymph glands, and subsequent reinjection of these glands. 1) First group of animals (glands removed at three days after the initial immunization); 2) second group (glands removed after seven weeks); 3) third group (control); ↓) revaccination.

lymph glands in the total immune response of the organism was comparatively small, and always the main organ responsible for immunogenesis of such a system were the lymph nodes remote from the site of the first injection of antigen. However, the completeness of the reorganization of the response of the latter during primary immunization depends on the presence at this time in the body of regional lymph nodes. This conclusion follows from a comparison of the titers of antitoxin of animals of the first and second groups, and leads to the same conclusion as was reached from the first experiment.

An extremely important part of the problem is the interaction between the nodes. Previously [1, 7] we have suggested that stimulation of the immune reorganization of the remote lymph nodes is due to colonization of the latter

As can be seen from the drawing, the early removal of the regional lymph glands caused a considerable reduction in the immune response to the subsequent injection of the antigen, but later removal produced no marked effect. The significance of the distinction was confirmed statistically.

Results of the last experiment confirmed the important role of the regional lymphatic glands in the reorganization of the immune response of the whole body, and enabled us to appreciate their double role: they act as an organ directly producing an antibody, and as a stimulator of the immune response of the removed lymph glands. Indeed, a comparison of the titers of antitoxin in animals of the second and third groups (see figure) shows that when the second injection was given, the relative part played by the regional

by sensitized lymphoid cells produced by the regional nodes. As Harris et al. [18] and Wesslen [24] showed, the regional lymph nodes liberate into the circulation cells capable of elaborating antibodies. It is also known that labelled lymphocytes transferred to another animal are found chiefly in the lymph nodes and in the spleen [13, 16]. The ability of the transferred lymphoid cells to survive in the host (particularly in the spleen and in the lymph nodes) and to function actively in it has been demonstrated by numerous experiments with "radiation Chimeras" [14, 19, et al.], and by tests with the so-called Topley phenomenon [17, 22, 23 et al.]. At present, many investigators consider the migration of lymphocytes from one organ to another to be of considerable importance in connection with many physiological and immunological processes [10, 11, 25, 26]. There are also indications which favor a humoral-endocrine relationship between different parts of the lymphatic apparatus [20].

Therefore, both our own and published reports concur in describing the lymph nodes as a single functional system interconnected by special functional ties. The problem of the nature of these ties in relation to immunogenesis requires further study.

SUMMARY

Removal of the regional lymph nodes after primary immunization with tetanus toxoid reduced the response of other lymph nodes to the subsequent injection of antigen. It is suggested that the stimulating effect of the regional lymph nodes is due to migration of sensitized lymph cells to the other nodes.

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