REVIEWS

The Past, Present, and Future of Research on the Regulation of Nonlymphoid Cell Proliferation by Lymphoid Cells

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Reviewed are data on the capacity of T lymphocytes to stimulate or inhibit nonlymphoid cell proliferation, on the correlation of this capacity with the activity of respective immunoregulatory cells (T helpers and suppressors), and on the possible cell-mediated and humoral mechanisms through which lymphocytes execute their morphogenic function.

Key Words: *proliferation; lymphoid regulation; morphogenetic function; immunoregulatory cells*

The regulation of nonlymphoid cell proliferation by lymphoid cells is central to the broader theory of the regulation by these cells of growth processes in health and disease. The history of studies on this theme may be divided into two periods. During the first period, research efforts were focused on exploring the so-called trophic function of leukocytes, whereby proliferating cells are supplied with nutrients liberated from leukocytes upon their destruction. Subsequently, emphasis was placed on the morphogenetic function of live lymphocytes, associated with their ability to interact with other cells and to produce biologically active substances and growth factors.

As this review is devoted to the morphogenetic function of lymphocytes, we will only briefly discuss four publications of the first period, since they laid the foundation for lines of research that made important contributions to our knowledge of how lymphocytes regulate cell division [2,3,6,28].

The doctrine of lymphocyte trophic function was founded by the well-known French surgeon Alex Carrel [29]. He was the first to call attention to the fact that culturing white blood cells in protein-free media enriches these with substances of protein nature (trephones) and makes them suitable for culturing cells taken from other, more "capricious" tissues that usually require the addition of embryonal extracts to the medium for their proliferation. Carrel believed that trephones enter the culture medium from disintegrated neutrophils.

Substantial contributions to the trophic function doctrine were made by Khrushchov and coworkers [21], who showed that the greatest capacity for stimulating the proliferation of cultured cells was possessed by culture media (leukocytic sera) containing more live than dead cells, irrespective of whether the cells were neutrophils or lymphocytes. These investigators also called attention to the ability of leukocytic sera to suppress, cell division in culture under certain conditions and pointed out the special stimulatory properties of leukocytes contacting cultured cells. The next stage in the evolution of this doctrine was marked by the discovery that lymphocytes making contact with dividing cells *in vivo* pass some of their nuclear substance on to them. That this is so was indicated by the transfer of ^{3}H-thymidine from the lymphocytes into which it had been incorpo-

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rated to dividing cells, in particular hepatocytes of a regenerating liver [27]. The morphogenetic nature of the influences exerted by lymphocytes was best grasped by investigators who observed therapeutic effects following the transplantation of live lymphocytes or thymic tissue to animals with signs of runt disease, which can result from immune deficiencies of various origin [32].

The further development of research on lymphoid regulation of nonlymphoid cell proliferation with special reference to the morphogenetic function of lymphocytes was intimately linked with the extremely rapid progress of noninfectious immunology, first and foremost with advances in the study of transplantation immunity, immunological tolerance, and autoimmunity. Investigations designed to clarify the cellular basis of these phenomena demonstrated that the cells chiefly responsible for their occurrence are lymphocytes, and that the activity of the immune system as a whole is of paramount importance for the maintenance of homeostasis in the body. The groundwork was laid for a study of the role the immune system plays in eliminating the consequences of injuries and in preserving the structural integrity of the organism [2,3,25,28] - its "morphostasis" [28].

Our research using various approaches [2] led us to the conclusion that attention should be focused on the function of lymphocytes during the regeneration of different organs, because the autoimmune processes presumed to go on in regenerating organs should, as usual, involve lymphocytes [2,3]. To demonstrate the morphogenetic function of these cells, we chose animal models of liver regeneration and compensatory kidney hypertrophy [7,8]. We found that the adoptive transfer of lymphoid splenic cells (splenocytes) from partially hepatectomized or unilaterally nephrectomized donor mice to intact syngeneic recipients resulted, 48-49 h posttransfer, in augmented proliferative activity in their respective organs. As has been established by immunologists, donor lymphocytes antigenically identical to recipient lymphocytes continue to perform their inherent function(s) in their new host. The failure of lymphocytes from intact or sham-operated mice to stimulate cell division in the recipients and the fact that killed lymphocytes from operated mice lost the capacity for such stimulation indicated that the live lymphocytes from operated donor mice were responsible for the observed stimulation of nonlymphoid cell proliferation in the recipients [7,8]. The viability of grafted lymphocytes was demonstrated using a chromosome label. These cells remained viable for at least 14 days [31].

The above investigations marked the beginning of the second period in the work on lymphoid regulation of nonlymphoid cell proliferation by setting the stage for a new line of research. They immediately attracted attention and were followed by a series of studies that both increased the number of organs where regeneration was observed and broadened the range of processes in which lymphocytes were found to be able to stimulate cell proliferation in various organs or tissues. Thus, lymphocytes were shown capable of stimulating cell division in a regenerating small intestine, skin and hematopoietic tissue, a pathologically changed liver and skin, a hypertrophic and pathologically changed kidney, and in animals with hypertrophic erythropoiesis or with a transplanted syngeneic tumor - that is, in damaged organs with high regenerative potential and in tissues with high proliferative activity [5,16,18,22-25,30,33,34]. The stimulation of cell proliferation in those studies was a true one, for the rise of the mitotic index was accompanied by an increase in the number of DNA-synthesizing cells, a substantial accumulation of mitoses in response to administered Colcemid, an increase in the number of cells in structural units of the recipient's organ (e.g., in intestinal crypts) or of cells (e.g., reticulocytes when the mitotic index of erythroblasts was high) reflecting the terminal differentiation stage of dividing cells, and by an increase in the weight of the organ or in the number of cells cultured from it [3,5,18,35].

Of special note is the ability of lymphocytes to determine the degree and type of nonlymphoid cell differentiation [5,18,20]. When splenocytes from a donor with resected small intestine or massive blood loss were transferred to an intact recipient, they reproduced in it, respectively, the mitotic pattern in the regenerating intestine and morphological markers of reparative erythropoiesis [5]. The transfer by lymphocytes of these and other characteristics of reparative processes prompted us to introduce the concept of "regenerative information transfer."

Several aspects of morphogenetic lymphocyte activity were clarified by knowledge of the timing of cell cycles and regenerative processes in various organs. Lymphocytes acquire an ability to stimulate cell proliferation long before DNA synthesis is initiated in the regenerating organ (e.g., 4 to 17 h after hepatectomy) and lose this capacity when cell division in the organ is at its height (44-49 h after the operation).

The foregoing suggests that morphogenetically active lymphocytes are implicated in triggering DNA synthesis and become inert once they have

completed their mission [4,7]. The time taken by lymphocytes to acquire a proliferation-stimulating capacity depends on which organ has been operated upon, mainly because the prereplicative period varies from one organ to another; for instance, it is shorter in a resected liver than in a resected kidney [7,8]. For this reason, the effect of active lymphocytes in the recipients can only be detected after the interval between the transfer of these cells and the completion of the mitotic cycle by cells of the particular organ [2-4,6,7,25]. These intervals range, for example, from 44-49 h in mice with resected liver to 4 days in mice with massive blood loss [5,7]. It has been shown that the cells responsible for morphogenetic activity are mature T lymphocytes [9,16,17]. Splenic T lymphocytes of partially hepatectomized mice treated with specific anti-T-lymphocyte sera during the period of their highest morphogenetic activity were found to lose this activity, whereas a similar treatment of B lymphocytes did not affect splenocyte activity [9]. Although the evidence that morphogenetic function and regenerative information transfer are accomplished by T lymphocytes is overwhelming, it must still be noted that they perform this function and transfer more effectively in cooperation with macrophages, as was shown most clearly in experiments with regenerating hematopoietic tissue [14].

Sensitivity to the stimulatory properties of lymphocytes was displayed by cells of unoperated and even more of operated recipient organs, mainly of organs homologous to the respective organs operated upon in the donors (and also by lymphoid cells both *in vivo* and *in vitro* culture) [3]. In other words, the proliferation-stimulating activity of lymphocytes from operated animals was predominantly organ-specific. The degree of morphogenetic activity exhibited by splenic lymphocytes during the postresectional regeneration of, for example, hepatic or renal tissue was directly related to the degree of tissue deficit created by the operation [3,8]. Thus, lymphocytes from donor mice in which 25% of the renal or hepatic tissue had been removed, unlike those from mice that had lost 50% of the renal or 70% of the hepatic tissue, failed to stimulate (under otherwise equal experimental conditions) cell proliferation in the recipient organ [8,10], but a second operation performed in the same mice to resect another 25% of their hepatic tissue when liver regeneration had been completed (20 days after the first operation) was followed by the emergence of morphogenetically active lymphocytes in the recipients [10]. Lymphocytes, therefore, possess a peculiar memory by virtue of which two operations widely separated in time can together elicit heightened morphogenetic activity of these cells, whereas neither of them can do so alone.

The enhanced ability of splenocytes from operated animals to stimulate nonlymphoid cell proliferation is associated, directly or indirectly, with an increase in the number of morphogenetically active lymphocytes in the body as a result of either their introduction from the outside or their endogenous accumulation [11], with a consequent rise in the proportion of these cells relative to that of lymphocytes with proliferation-suppressing properties [13].

Consideration of the functions performed by proliferation-suppressing lymphocytes and proof of their existence in the body are important for demonstrating the validity of our concepts regarding the regulatory role of lymphoid cells [2,6]. The term "regulation" implies not only stimulation of proliferation but also its termination by antagonistic cells. In this context it seems appropriate to discuss first the influence exerted on cell proliferation by lymphocytes of intact animals, which appear to sustain cell proliferation at a particular level in the organism. According to the available evidence, thymocytes from normal animals suppress the proliferation of enterocytes in crypts of the small intestine in nonoperated recipients [19,20] and weight gain by a hypertrophic kidney [35] to varying degrees depending on the dose and viability of transferred thymocytes. Thymocytes killed by heat were not suppressive [19]. Splenic lymphocytes from intact animals suppressed hepatocyte proliferation in the liver of recipients in some experiments and slightly stimulated it in others [9,11]. However, both thymocytes and splenocytes invariably restored the level of cell division reduced by various interventions - in the cornea of partially hepatectomized animals, in the liver of irradiated recipients, and in cryptal enterocytes of the small intestine in thymectomized animals; they also promoted the healing of skin wounds and raised to normal the compensatory renal tissue growth in irradiated recipients [2,4,9,16,23,33].

Proliferation-suppressing lymphocytes exist in the body to dampen the proliferative wave in a regenerating organ. In particular, they appear in the spleen during liver regeneration at a time when mitotic activity in hepatocytes is on the rise and prevent further hepatic cells from entering mitosis without interfering with its completion by cells that have already received an impetus to divide [11]. This lymphocyte population also appears to be implicated in rendering some organs, including the

submandibular gland and spleen, almost incapable of regeneration. Operations such as unilateral sialadenectomy and partial splenectomy are regularly followed by the appearance of lymphocytes with proliferation-suppressing properties [4,11]. After adoptive transfer, these cells inhibited the proliferation of hepatocytes in a regenerating liver, corneal epithelial cells, epithelial cells of the small intestine, and antibody-producing lymphocytes [4,25]. The degree of such nonspecific suppression depended on the dose of transferred lymphocytes and on how much the level of endogenous suppressors had been lowered by the operation [11]. In the examples just cited, cell proliferation was inhibited by suppressor T cells, as was indicated by the recorded accumulation of these cells in that part of the spleen remaining after splenectomy [3,6].

The studies mentioned above suggest that the activity of lymphocytes with stimulatory and suppressive properties largely depends on their ratio in the body. Evidence for this is provided by experiments in which the population of T helpers or suppressors is artificially reduced or increased. Elimination of T suppressors with an antiglobulin directed at these cells resulted in a generalized elevation of mitotic activity in a number of organs (liver, kidney, spleen, bone marrow, small intestine, cornea); when, on the other hand, the suppressor T-cell population in the spleen was increased by inducing immunological tolerance, the mitotic activity of tissues with a high proliferative potential (bone marrow and spleen) sharply decreased [17]. Analyses of how the ratio between the populations of T helpers and suppressors in the spleen of operated mice and the behavior of these cells in immune reactions are altered support the conclusion that reparative processes are accompanied by the accumulation of helper T cells in organs with a high regenerative potential and of suppressor T cells in those with a low potential for regeneration [4,7,25]. During the period when the morphogenetic activity of splenocytes in unilaterally nephrectomized mice was elevated, the number of lymphocytes bearing a helper T marker ($FcIgM^+$) was significantly increased, whereas changes in the numbers of lymphocytes with a suppressor T marker (FcIgG) and of immature lymphocytes (spontaneous rosette-forming T cells and TDT^+ cells, i.e., those bearing the marker terminal deoxyribonucleotidyl transferase) were insignificant [13].

What role, if any, these immature lymphocytes play in morphogenetic activity remains to be elucidated. The immunoregulatory T cells, i.e., helpers and suppressors, certainly play a major role in this respect. The data at hand indicate that mature T lymphocytes perform their morphogenetic function in the same ways as they do their immunological function - through cellular contacts and by means of the lymphokines they produce.

Electron microscopic examination of regenerating livers at different times after partial hepatectomy (with the removal of two-thirds of the liver tissue) showed that as early as 4 h after the operation many lymphocytes were in close contact with hepatocytes and liver macrophages; after 17-48 h, the number of lymphocytes in contact with hepatocytes was increased, as was the length of these contacts. In some instances, so-called microtunnels through which morphogenetic (substrate) information can be transferred were seen to appear at the contact sites. Such contacts were maintained throughout the period of the proliferative wave (from the time of its preparation to its completion), i.e., for 72 h after the operation [12]. Similar observations were made earlier for regenerating ulcer-affected stomachs [1] and can probably be extended to other organs as well.

The nature of the substrates transferred in such cases is still unknown. It may be that various lymphokines are transferred, since cultured splenocytes from partially splenectomized or unilaterally nephrectomized mice have been shown to produce humoral factors of protein nature which, like lymphoid cells themselves, alter the proliferative activity of other cell types [26]. The humoral factors recovered after culturing lymphocytes from mice with resected spleen or liver were capable of suppressing the proliferation of hepatocytes in a regenerating liver and of corneal and small intestine epithelial cells; in contrast, splenocytes from unilaterally nephrectomized mice produced a factor stimulating the proliferation of renal tubule epithelial cells [26]. It is significant that these factors can also alter the level of immunological reactions, which suggests that the factors with morphogenetic activity may be identical to the lymphokines involved in the immune response. Moreover, the behavior of morphogenetically active lymphocytes resembles that of lymphocytes in immune processes. In executing their morphogenetic functions, they can act as regulators and effectors in interacting with nonlymphoid cells of the target organ to alter their proliferative activity. However, in the immune response to a thymus-dependent antigen, immunoregulatory cells interact with other lymphocyte subsets - with the effectors of this response.

On the strength of all the evidence, lymphocytes are bifunctional, as were their phylogenetic predecessors, but during the early periods of the evolution of the animal kingdom, the morphoge-

netic function was better developed than the defensive function. In the course of time, as the latter function became increasingly more complicated, the range of morphogenetic lymphocyte activity narrowed and became restricted to cytogenesis. Nevertheless, the morphogenetic function persisting in higher animals plays important roles in growth processes, physiological and reparative regeneration, and tumorigenesis. Inadequate knowledge or underestimation of the mitogenetic and mitostatic functions performed by immunoregulatory cells hinders the unraveling of immunobiological mechanisms involved in tumor formation. The theory of immunological surveillance over this process has not received unanimous support. Immune deficiency can either promote the neoplastic process or (e.g., after neonatal thymectomy [15]) prevent it. This seeming discrepancy can be accounted for by invoking the concept of morphogenetic activity. Eliminating the T lymphocytes or drastically reducing their population deprives the organism of proliferation regulators, especially stimulators.

Future research on the topic of this review should concentrate on unraveling the subtle mechanisms through which the morphogenetic activity of lymphocytes is realized, because only then will we have a complete understanding of such crucial processes as regeneration and malignant growth.

REFERENCES

1. L. I. Aruin and O. L. Shatalova, *Arkh. Pat.*, № 8, 11-17 (1981).
2. A. G. Babaeva, *Immunological Mechanisms Regulating Regenerative Processes* [in Russian], Moscow (1972).
3. A. G. Babaeva, *Regeneration and the System of Immunogenesis* [in Russian], Moscow (1985).
4. A. G. Babaeva and V. V. Arsent'eva, *Byull. Eksp. Biol. Med.*, **112**, № 8, 169-170 (1991).
5. A. G. Babaeva and E. I. Belan, *Ontogenez*, № 2, 125-131 (1988).
6. A. G. Babaeva and E. A. Zotikov, *Adaptive Growth and Proliferation and Their Disorders: Immunological Aspects* [in Russian], Moscow (1987).
7. A. G. Babaeva, N. A. Kraskina, and L. D. Liozner, *Tsitologiya*, № 12, 1511-1520 (1969).
8. A. G. Babaeva, N. A. Kraskina, and L. D. Liozner, *Byull. Eksp. Biol. Med.*, **75**, № 2, 78-80 (1973).
9. A. G. Babaeva, N. A. Kraskina, and N. V. Yudina, *Byull. Eksp. Biol. Med.*, **89**, № 1, 69-70 (1980).
10. A. G. Babaeva, V. G. Nesterenko, and N. V. Yudina, *Byull. Eksp. Biol. Med.*, **93**, № 6, 98-99 (1982).
11. A. G. Babaeva, N. V. Yudina, and T. A. Druzhkova, *Ontogenez*, № 6, 573-581 (1988).
12. A. G. Babaeva, V. A. Shakhlamov, and N. V. Yudina, *Byull. Eksp. Biol. Med.*, **117**, № 2, 176-179 (1994).
13. A. G. Babaeva, A. N. Shutko, N. N. Shatinina, *et al.*, *Arkh. Pat.*, № 1, 62-64 (1993).
14. E. I. Belan and L. L. Golovkina, *Byull. Eksp. Biol. Med.*, **118**, № 9, 287-290 (1994).
15. S. N. Bykovskaya and E. V. Gruntenko, *T Lymphocytes in Antitumor Immunity* [in Russian], Novosibirsk (1982).
16. I. I. Dolgushin, *Pat. Fiziol.*, № 6, 30-32 (1978).
17. N. A. Kraskina, E. M. Veger, N. M. Gutorova, *et al.*, *Immunologiya*, № 5, 39-45 (1987).
18. T. B. Timashkevich, G. V. Kharlova, and N. V. Yudina, *Byull. Eksp. Biol. Med.*, **97**, № 3, 352-353 (1984).
19. V. A. Trufakin and A. N. Shmakov, *Arkh. Anat.*, № 5, 73-79 (1990).
20. V. A. Trufakin and A. N. Shmakov, *Vestn. Akad. Med. Nauk SSSR*, № 12, 23-28 (1991).
21. G. K. Khrushchov, *Role of Blood Leukocytes in Regenerative Processes* [in Russian], Moscow (1945).
22. L. Ya. Shipova, V. S. Poltoranina, and A. P. Suslov, *Byull. Eksp. Biol. Med.*, **93**, № 6, 99-101 (1982).
23. A. N. Shmakov and V. A. Trufakin, *Tsitologiya*, № 12, 23-28 (1989).
24. A. P. Yastrebov and M. V. Popugailo, in: *Mechanisms of Regulation in the Blood System* [in Russian], Krasnoyarsk (1978), pp. 224-226.
25. A. G. Babaeva, *Life Sci.*, **172**, 121-128 (1989).
26. A. G. Babaeva, T. A. Druzhkova, and N. V. Udina, *Monogr. Dev. Biol.*, **23**, 223-229 (1992).
27. B. J. Bryant, *Exp. Cell Res.*, **27**, № 1, 70-79 (1962).
28. R. G. Burwell, *Lancet*, **2**, 69-74 (1963).
29. A. Carrel, *J. Exp. Med.*, **36**, 385-391 (1922).
30. J. Fidler, *Biomedicine*, **32**, № 1, 1-3 (1980).
31. M. Fox and G. E. Wahman, *Invest. Urol.*, **5**, № 6, 521-538 (1968).
32. G. F. Loutit, *Lancet*, **2**, № 7265, 1106-1108 (1962).
33. M. E. Pliskin and R. T. Prehn, *J. Reticuloendothel. Soc.*, **17**, № 5, 290-299 (1975).
34. R. T. Prehn, *J. Reticuloendothel. Soc.*, **10**, 1-16 (1971).
35. B. Radocevic-Stasic and D. Rukavina, *Period. Biol.*, **81**, № 2, 151-152 (1979).