REVIEWS

Involvement of Natural Killer Cells in Endogenous Biological Retranslation

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Analysis of functional properties of natural killer cells displayed in reactions of natural cytotoxicity and noncytotoxic regulatory intercellular interactions suggests that this population of lymphocytes is involved in endogenous biological retranslation. In the immune system, retranslation is the production of regulatory immunoactive cytokines by a cell, cellular complex, or functional complex. The substances produced are identical to those affecting these structures. Various forms of endogenous biological retranslation in humans and higher animals, as well as its phylogenetic and ontogenetic manifestations (on the basis of noncytotoxic regulatory interactions of natural killer cells with cells of lymphoid or nonlymphoid nature) during evolution of the complex of immunobiological surveillance are considered. The axiomatic basis of retranslation realized through the system of natural cytotoxicity was established. Prospects for application of the methodology of endogenous biological retranslation to experimental and clinical studies of functioning of natural killer cells are considered.

Key words: *natural killer cells, endogenous biological retranslation*

The system of natural cytotoxicity (NCT) functions as a universal mechanism controlling proliferation and differentiation of cells in humans and higher animals [16,52,58,72]. In evolution, this system is considered to be the most ancient factor of immunobiological surveillance [38,43,57]. The factors mentioned above account for the increasing interest in this cellular phenomenon [43,50,58,59,66,76, 80,113].

The resistance to viral infections and tumor growth [55,58,80,113], as well as the efficiency of processes of tissues rejection and regeneration [86, 92,112] and elimination of functionally aged cell forms [12], are thought to be related to the level of activity of natural killer (NK) cells.

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NK cells produce a wide range of immunoactive cytokines [25,42,67,78,84,85,95]; interact with lymphoid and nonlymphoid cells [28,29,34,35,64, 81,90,105]; and exert direct cytotoxic effect on T and B lymphocytes, several subpopulations of bone marrow cells, adherent peritoneal cells, and transformed fibroblasts [50,56,68,91]. NK cells compose an important element of regulation of hematopoiesis, lymphopoiesis, and immunogenesis [40,54,56, 64,100].

Blockade of maturation of NCT effectors, defects of lymphocytic enzyme systems responsible for programming and realization of several stages of the lytic cycle, and changes in the regulatory effects of neuroendocrine factors result in the development of immune deficiency of NK cells. This is accompanied by violation of functional interrelations in the immune system, activation of autoaggression mechanisms, and formation of total immune defi-

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ciency [17,18,37,44,46,73,88, 89,108,116,117]. Pathogenesis of immune dysfunction developed during the chronic fatigue syndrome is considered to be closely related to inefficient realization of the cytotoxic potential of NK cells directed against virus-infected target cells [26,32,113].

In spite of long-standing studies of NCT [17,56, 58,59,66,76,113], medicine has no immunotherapeutic drugs displaying selective prolonged activity toward NK cells. Experimental and clinical approaches capable of restoring not only cytotoxicity of several effector cells but also contact and distant interrelations in the NCT system (which were lost in immune deficiency) enabling normal immunobiological surveillance of this system under physiological conditions have not been designed [18,39,48, 60,61,101,102].

This is probably due to the fact that intrinsic regulatory mechanisms of the system (including autocrine regulation of NK cells) determining and maintaining a certain level of cell functioning under physiological conditions and realization of specific activity (without direct relationship to induced immunogenesis) remain unknown. In this aspect, cytotoxic and regulatory interactions mediated by NK cells received little attention in previous studies. There are nearly no data on the mechanisms responsible for the dynamic regulatory equilibrium in the complex of immunobiological surveillance. The extent to which NK cells function autonomously in immune reactions remains unclear. The ability of the NCT system for endogenous compensation and its differentiation and activation potentials could be inferred only the indirect methods.

Interferon (IFN)-α, IFN-γ, and interleukin (IL)-2 belong to immunoactive cytokines produced by NK cells stimulated by exogenous inductors or factors of homeostatic regulation [25,42,67,115]. These cytokines are the most important mediators controlling differentiation of NCT effectors and realization of their cytotoxic potential [23,79,93,103,111]. This confirms the existence of mechanisms of autocrine regulation of NK cells and suggests the involvement of NCT system in processes of endogenous biological retranslation (EBR). These processes probably provide the basis for noncytotoxic interactions of NK cells with lymphoid and nonlymphoid cells. Specific features of functional organization of NCT system enabling it to function as an endogenous biological retranslator that perceives and produces identical molecular signals, as well as the mechanisms controlling EBR through the NCT system, remain unknown. Their elucidation would noticeably expand our understanding of common principles of immunoregulation in the body. These principles are important for analysis of the pathogenesis and prognosis of diseases associated with development of the NK deficiency. Moreover, studies of specific features of functional organization of the NCT system would provide new possibilities for screening the agents and methods of selective immunocorrection (first and foremost, restoration of regulatory interrelations of the NCT system, which are important for maintaining the immune homeostasis).

EBR in Cellular Systems

There is a large body of postulates and data derived from experimental and theoretical studies which describes particularities and main principles of immunogenesis in higher animals and humans. EBR is rather the sole property of immunocompetent cells that received little attention and has no definite meaning. However, this property acquires a specific biological significance once analyzed in detail. This phenomenon is important not only for fundamental science; it also plays a considerable role in elaborating problems of immunoregulation and immunocorrection under experimental conditions and in clinical practice.

The ability of several effector cells to generate molecular signals (to produce immune mediators and regulatory factors) identical to those affecting the producer cell is well known. This property is typical of the most phylogenetically ancient nonspecific effectors involved in the immune response (monocytes/macrophages) and clonally selected T and B lymphocytes constituting the main components of specific reactions to antigenic stimulation.

IL-1α, IL-1β, IL-3, IL-6, IL-8, IL-10, macrophage and granulocyte-macrophage colony-stimulating factors, tumor-necrosis factor-α, and IL-1 receptor antagonist belong to immunoactive compounds perceived and produced by the cells of the monocyte and macrophage types [3,45]. T lymphocytes differ in their abilities to respond to (and produce) IL-2 and IFN-γ. The intensity of the effector stage of the immune reaction is associated with activities of these agents [4,22].Tumor-necrosis factors of the α and β types, IL-6, and IL-9 also belong to regulators perceived and produced by these cells. B lymphocytes respond to (and produce) tumor-necrosis factor-α, IL-2, IL-6, and IL-10 [4,11].

The same molecular signal occurs at the input and output of the cell, cellular complex, or functional complex independently of temporal parameters, dynamics of reaction, and microenvironmental conditions providing perception of a mediator and initiation of molecular mechanisms of its

synthesis and secretion. This process is EBR of the mediator, which affects the cell, cellular complex, or functional complex.

The majority of recent data do not describe or analyze (in the context of EBR) intercellular interactions forming a branched network of cytokine regulation of the immune response. Special studies thus far were not performed. The functional properties of monocytes and macrophages mentioned above are commonly considered in the context of autocrine regulation of cellular activity. This suggests a primary role of spontaneous or induced (to a greater extent) generation of the molecular signal, rather than the primary role of its perception [3]. Corresponding characteristics of T and B lymphocytes are evaluated with respect to the paracrine control of immunogenesis under forced effects [4,11] or to regulation of maturation of immunocompetent cells [21,22]. Therefore, system contains an acceptor cell, which is different from a producer cell. The primacy of functions is also attributed to mediator production.

In contrast, the EBR theory establishes the primacy of reception of a molecular signal (in the methodological aspect and not in aspects of general biology and evolution). This theory assumes that the same effector functions as the acceptor and the producer of immunoactive compounds. In this case, the effector itself (autocrine regulation) or the cell of a different nature (paracrine regulation) can play a role of the target cell of regulatory effects. The directionality of effects depends on the dynamics of the process and actual microenvironmental conditions (e. g., the distance of intercellular interactions and possibility for the mediator to enter the circulation system).

These data indicate that EBR processes (whose essence remains unknown) can proceed in the complex of intercellular exchange responsible for realization of the immune response of certain level in higher animals and humans. The problem of EBR processes remains unsolved probably because of particularities of immunogenesis that make detecting EBR in the immune system difficult. Numerous interrelations between intensively functioning cellular elements of the system and a high heterogeneity of the subpopulation of immunocompetent cells (at the known plasticity of immune processes) make physical recording of signal translation (its generation, transition, and reception) nearly impossible.

Thus, analysis of EBR in the immune system can be performed only by indirect means. In turn, this requires elaboration and use of principally new conceptual approaches to regulatory interactions. These approaches must not be limited by the algorithm of the notion of "regulation". This notion assumes determination of three parameters recorded: the initial level of realization of certain cellular function, the intensity of the regulatory signal (and the fact of its action), and the changed level of realization of the same function in the same cell. The use of the EBR methodology in immunology, immunobiology, and general biology would significantly change fundamental prerequisites for any analysis. Our studies of this problem indicate that it is not necessary for EBR to affect considerably the parameters of lymphocyte functions registered by traditional methods of analysis. However, EBR processes may be responsible for the establishment of particular limitations in the systems of intercellular exchange contributing to a more efficient performance of the function of signal translation by the cell [19].

Therefore, further refinement of knowledge promoting the understanding of evolutionary principles of immune processes and their role in the system of regulatory intercellular interactions responsible for reactions of surveillance and control is associated with widening of the methodological basis for fundamental and applied studies in the fields of immunoregulation and immunocorrection. This goal may be reached by applying phenomenology and methodology of EBR to the analysis of particularities and main principles of functioning of the immune system. In the immune system, EBR is considered to be a realization of capabilities of an effector lymphocyte, other immunocompetent cell, or cellular complex for reception and production of identical immunoactive compounds.

Forms of EBR in Humans and Higher Animals

The interrelations showed in Fig. 1 allowed us to describe the possible forms of EBR realized by a single effector cell (*1*), cellular complex (*2*), or functional complex (*3* and *4*). In the latter case, the assignment of experimental or clinical situation to EBR processes does not depend on the nature of additional compounds involved in realization or regulation of the activity of the complex. Formation of the same molecular signal at the cell input and output (substance *B*) is the only important factor.

Such an analysis shows that translational properties of cells of the immune system have long been known (as applied to certain cellular functions). Moreover, these properties form the basis of methods for the synthesis of particular immunoactive compounds that are widely used in clinical practice. However, the translational nature of the process remains to be established.

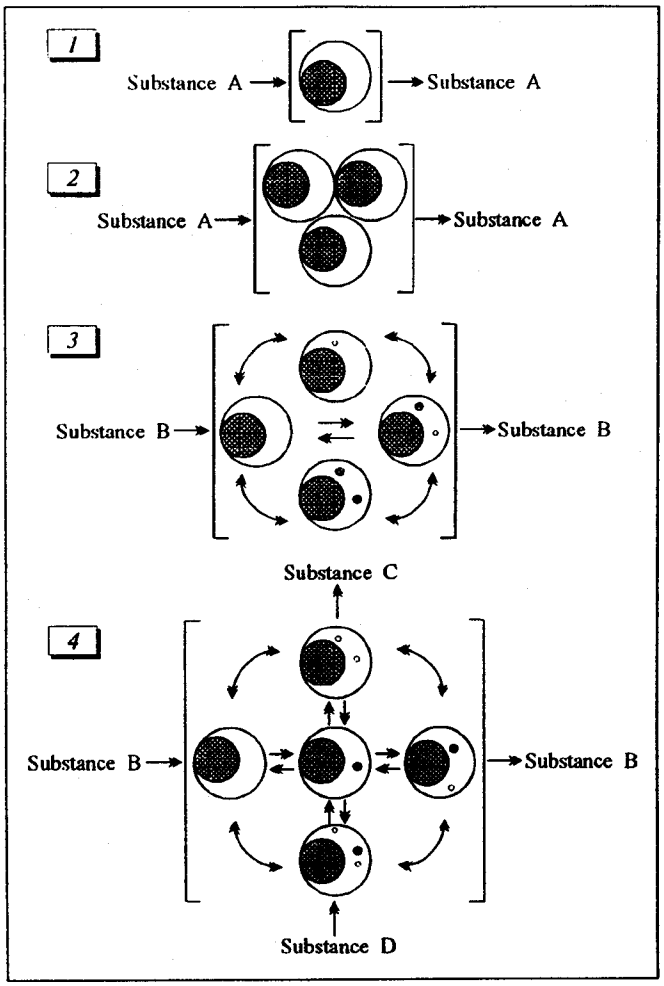

Fig. 1. Possible forms of endogenous biological retranslation in cellular systems.

Here, we mean a well-known and widely used in extended biotechnological industrial processes phenomenon of priming of IFN production, activation of IFN genesis by preliminary treatment of producer cells with IFN preparations [65,77,99]. The priming properties of IFN-γ and IFN-α were observed in the system of IFN-γ production. In spite of this fact, it is important to note that optimal priming doses of IFN-γ were considerably lower than such doses of IFN-α [7]. These data indicate that EBR processes are physiologically regulated by the state of immunoreactivity, which is characterized by considerably lower inducing parameters than forced immunogenesis. The requirement for the inductor of IFN (Fig. 2) allowed us to assign priming of mediator production to the variant *4* (Fig. 1).

The reaction of NCT may be assigned to the same form of EBR. During this reaction, production of IFN (which is also pronounced at pretreatment of effector lymphocytes with IFN [19]) is recorded [14,20]. The target cell undergoing cytolysis mediated by NK cells plays a role of the IFN inductor (Fig. 3).

Both in the case of obtaining a priming effect and in the NCT reaction, the dynamics of the process [1,6] and the titer of the mediator produced [14,20] are such that (with a high probability) the translated regulatory factor is not used in the complex of autocrine regulation only. It reaches various cellular forms (other than producer cell), e. g., target cells of the lytic effect, effector lymphocytes, and numerous auxiliary cellular elements responsible for NCT reactions and IFN production [19].

Therefore, the distance of intercellular interactions realized via translated mediators becomes an important characteristic allowing us to differentiate EBR processes and realization of the potential of autocrine regulation. It determines the distribution of these processes beyond the immune system, because any biological system whose effectors can produce compounds identical to affecting compounds and reaching target cells differed from the producer effectors may be involved in EBR processes.

The discovery of translational principles of the priming phenomenon of IFN production and NCT reaction required a certain conceptual theory. In contrast, authors of studies conducted at the end of the 1980s and at the beginning of the 1990s came to the description of EBR processes in cellular systems.

Human recombinant (r) IL-1α and IL-1β were shown to increase (in a dose-dependent manner) the levels of IL-1β mRNA in endothelial cells *in vitro* and rIL-1α in nonadherent mononuclear cells (IL-1α mRNA and IL-1β mRNA). They stimulated the production of IL-1 *in vitro*. In doing so, rIL-1α induce the synthesis of IL-1β in human mononuclear cells [41,110]. Human rIL-1α administered to rabbits induces the production of their own IL-1 [41]. However, the finding of the ability of functional complexes involved in IL-1 production to reproduce the affecting factor did not become a logical result of experimental studies.

During adaptation of the body to physical load, neutrophils can operate as protease vectors by acting on the sites of tissue remodeling and localizing tissue processes of regeneration. These data did not lead to formulation of certain concepts and analysis of translational principles of the phenomenon [97]. In this case, however, a certain type of transport of biologically active compounds (rather than translation of signal inducing or regulating cellular activity) should be considered and discussed.

In the mid-1990s, biological retranslation became a conceptual problem as applied to particula-

rities of interspecies relations in an ecosystem mediated by inductors of genetic diversification of prokaryotes. Prokaryotes perceive "an integrated signal, which is considered to be dangerous for reproduction of generation and/or realization of the program of the productive phase of the life cycle" and generate a universal response, molecules "stimulating the process of genetic diversification and selection of mutant forms of these and other microorganisms" [8]. Intensive studies of compounds similar in composition and functions to exometabolites of cyanobacteria [8,36,74,75] allowed the revealing of several specific features of general translation important for the understanding of organization of endogenous translating systems. This concerns a rational confinement of the specialized cellular activity, which probably contributes to microenvironmental conditions beneficial for translation of immunoactive mediators.

The data discussed above show that the EBR theory results from a logical extension of original studies suggesting the possibility for the corresponding processes in cellular systems. However, the translational essence of the processes was not considered in the context of EBR [7,41,65,77,99,110]. In several studies, these principles were not believed to have a translational nature [97] or to be realized endogenously [8]. Thus, the evidence for the possibility for reproduction of signals of affecting cytokines in cellular systems did not lead to methodological concepts of EBR. This idea remains unstudied theoretically and unproved experimentally. It is not used for elaborating the functional purposes and resolving practical problems of immunoregulation and immunocorrection.

Here, we generalized for the first time the data on systemic analysis of EBR and the role of translational processes in the establishment and maintenance of physiological level of intercellular exchange in the immune system of the body. This is an attempt to call attention to a new methodology contributing to the understanding of the origin of controlling immunological reactions, their relation to noncytotoxic regulatory phenomena, and their role in the general system of cellular interactions forming the dynamic immunoregulatory equilibrium [19].

EBR Realized Through the NCT System in the Evolution of the Complex of Immunobiological Surveillance

EBR presumably provides intercellular interactions in the network of cytokine regulation of physiological immunogenesis. It is not occasionally that its development is directly related to evolution of the

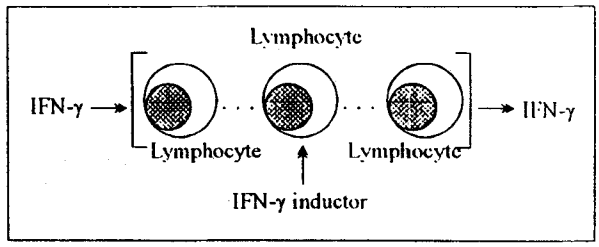

Fig. 2. Priming of production of interferon (IFN) in the context of endogenous biological retranslation.

complex of immunobiological surveillance. In addition to strong cytotoxic, antitumor, and antiviral properties, population of NK cells performs important regulatory functions in processes of hematopoiesis, lymphopoiesis, and immunogenesis. Some of these processes are not mediated by cytotoxic effects on target cells [33,40,68,105].

Evolution of the complex of immunobiological surveillance was accompanied by the appearance of highly specialized cellular forms and multifold extending the range of contact and distant interactions of NK cells with effectors of various origins. In higher animals and humans, it provided the formation of functional system having cytotoxic and regulatory properties. These properties allow performing surveillance functions and EBR under conditions identical to microenvironmental conditions and to effects of factors of exogenous induction and endogenous destabilization.

Let us suppose that EBR processes realized through the system of NCT (as well as NCT reactions) evolve phylogenetically from (or provide the basis for) noncytotoxic regulatory interactions of NK cells with cells of lymphoid and nonlymphoid nature. In this case, EBR may be attributed to primary (the most ancient) forms of intercellular exchange. Evolutionary analysis [9,15,43] shows that unicellular organisms have already actual requirements for arrangement of mechanisms that can provide transmission of the biological signal. Indeed, distinction is made possible by a contact with the object. The reaction (change in metabolism) proceeds after identification that assumes (in its turn) translation of information of surface receptors into the cell nucleus. In the cell nucleus, a correlation is made between perceived characteristics of the affecting object with the genetic program determining construction of own structures. Membrane structures must already display the potency to reproduce (in an encoded form) the characteristics of the affecting object performing the function of biological retranslation during transmission of information from the external medium to the nucleus. EBR can be assumed to develop in unicellular organisms

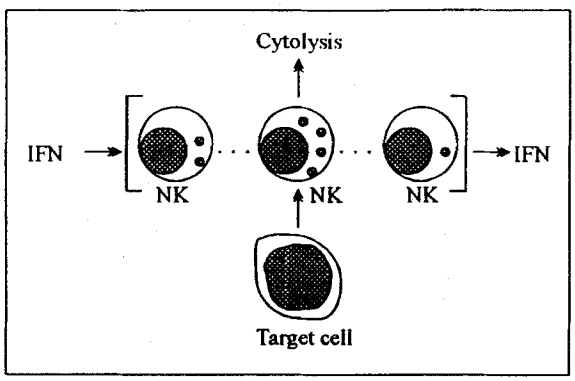

Fig. 3. Reaction of natural cytotoxicity in the context of endogenous biological retranslation. IFN (interferon), NK cell (natural killer cell).

(*Protozoa*) on the basis of distinction of genetically (antigenically) identical material. During the evolutionary process, EBR becomes more complex appearing in specialized forms and developing highly differentiated mechanisms. In further phylogenesis, EBR is transformed from the function of surface receptors to the function of individual cellular populations and cellular systems.

The NCT system has a key role in providing reactions of immunobiological surveillance in multicellular organisms and can be assigned as a variant of functional complexes that obtain several characteristics of endogenous retranslators during development of multicellularity. This forms the basis for involvement of NCT effectors into realization of EBR processes at the level of a highly organized biological object.

Phylogenetically, NK cells are more ancient than T and B lymphocytes [15,94]. Therefore, NK cells came into being earlier than specialized humoral regulatory factors of immunogenesis [43] and antibodies [9,38] during evolution. The NK activity in relation to allogeneic target cells mediated by primary immunocytes, hematocytes, and evolutional precursors of lymphocytes, celomocytes, is identical to that of mammals. It was found *in vitro* in tunicates [43,70], insects [43], crustaceans [43,98], mollusks [114], annelid worms (including sipunculids), roundworms, and flatworms [38,43,107]. Thus, the NK activity is retrospectively found in nearly coelenterates appeared at early stages of development of multicellularity. Before the appearance of specialized T lymphocytes, the functions of providing immunological memory are probably passed precisely to these cells that are designated as "living fossils" [51]. Expression of LFA-1 on the membrane of NK cells in humans is comparable only to that of T cells of memory, and exceeds markedly the number of such complexes on the surface of resting lymphocytes [87].

In embryogenesis that is generally similar to phylogenesis of higher animals, immunocompetent NK cells develop earlier than T lymphocytes, and probably participate in the regulation of hematopoiesis to the appearance of T lymphocytes [76]. Analysis of particularities of immune reactions in perinatal ontogenesis allowed us to assume that regulatory functions of NCT effectors are primarily (prenatally and in newborns) performed by synthesized (or translated) immunoactive cytokines (and not by cytotoxicity itself) that control processes of proliferation and differentiation of cells in a rapidly developing body. NK cells do not realize their cytotoxic potential up to the birth. Lymphocytes isolated from the umbilical cord blood are characterized by low NK activity (compared with cells of adult donors) [82,83,104] and lower capacities for the formation of effector-target conjugates and production of cytotoxic factor of NK cells and IFN-γ [83]. Cytotoxic activity of NK cells in newborns can be only slightly increased *in vitro* [104]. Therefore, its realization is inhibited. This contributes to immunoregulatory intercellular interactions under noncytotoxic translational regimen.

At the same time, processes of formation of the circulating pool of activated effectors of NCT (to the moment of birth) reach the providing level which corresponds to that in adults [53]. It sustains the basal activity of NK cells, which depends on inductive effects of environmental microbial factors during further evolution [2], and is retained under conditions of operative exclusion of regulatory effects of thymic hormones and microenvironment [50].

Irrespective of plasticity of this lymphocyte population, the NCT system is characterized by particular parameters of functioning which are sufficiently strongly specified. This indicates the existence of potent mechanisms of its internal regulation and a certain isolation from other populations of immunocompetent cells. Generation of cytotoxically active NK cells expressing the NK 1.1 marker in cell culture of mouse bone marrow proceeds without signals of exogenous cytokines [106]. The number of mature effectors of NCT varies slightly in the human peripheral blood [27,49,58]. The activity of NK cells in the bone marrow does not change with age [69].

Phylo- and ontogenetic manifestations of relative autonomy of NK cells in the reactions of immune surveillance and control presented here confirm a particular role of this population of lymphocytes in providing regulatory noncytotoxic intercellular interactions.

Realization of EBR through the NCT system seems to be more biologically preferable than trans-

lational processes performed by subpopulations of antigen-specific T and B lymphocytes and monocytes/macrophages. The possibilities for transmission of regulatory signal by cells involved in the forced immune response strongly depend on microenvironmental conditions and inductive component of immune reaction. Effectors of NCT display no high density of specific recognizing structures. They are not involved in antigen presentation (manifestation of a primary potency for biological retranslation during the specific immune response). These effectors express only the postulated NK receptor on the membrane [50] or realize the specialized activity in the absence of such a receptor [5]. Thus, NCT effectors is the least specific (in relation to their effects) population of lymphocytes. The level of its diversity is "relatively low and cannot be compared to clonal diversity of specific T cells" [15]. Effectors of NCT and the cells of monocyte/macrophage type are closely related in their origin [66,79]. The mode of operation of NK cells which are involved to a considerably lower extent (or nearly uninvolved) in the formation of the specific response is close to physiological mode. Thus, NK cells have genetically determined potency for realization of EBR processes that are not associated with forced immunogenesis.

Functioning of NK Cells under Conditions of IFN Translation

IFN plays a key role in the regulation of the intensity of maturation of NK cells from the bone marrow precursors, activation of cytolysis mediated by NK cells, control of recognizing and binding of target cells by effectors of NCT, and recycling of lympho-cytes [19,93,103]. This fact and the potency of NK cells for rapid production of IFN [25,67, 115] determine the possibility of considering the interrelations between NCT and IFN systems in the context of formation of unified morphofunctional complex of establishing and maintaining immunoregulatory equilibrium.

Indeed, physiological IFN response provides a certain level of IFN-dependent enzymes in mononuclear cells and in the plasma of healthy donors [30]. This indicates a permanent induction of IFN maintaining its constant background production in cellular microenvironment. The basal level of NCT is a likely manifestation (or consequence) of physiological IFN response. This level results from IFN-induced continuous differentiation of pre-NK cells to active forms that can bind and potently lyse the target cells [30,31]. Immunoactive IFNs are not found in the plasma under physiological conditions [30,31]. Nevertheless, they are found in all organs and tissues except for the central nervous system and the adrenal gland cortex and medulla [71].

Functionally active NK cells are also abundant practically in all peripheral compartments of the immune system. They realize particular functions in the lymphoid structures of the mucous membranes of the bronchi [24], stomach [10], and intestine [47, 62], in the palatine tonsils [109], mesenteric lymph nodes [47], and skin (NK-like AsGM1$^+$ cells [96]), controlling the growth of trophoblast in the composition of the decidual membrane *in vivo* (CD56$^+$ large granular lymphocytes [13]). NK cells are the major producers of IFN in the body. NK cells supply tissues and biological fluids with these mediators [25,67,115] and form (under conditions of physiological IFN response) polyfunctional network of intercellular exchange with an evolutionary determined potency for realization of EBR processes. The rational intensity of this network functioning is due to constant translation of IFN.

Let us suppose that the highly differentiated effectors of NCT (activated NK cells) circulate in the state committed to IFN production. These effectors can become unblocked (i. e., induced by target cells displaying regulating or lytic effects in the microenvironment) and exhibit the ability for synchronized production of the mediator. Only part of the cells translate constantly in a local and time-limited manner. However, this part of effectors (taking into account the abundance of NK cells in peripheral parts of the immune system described above) is sufficient for the induction of translational field of IFN that provides physiological basis for functioning of cytokine immunoregulatory network. Autocrine and paracrine components of intercellular exchange involving NK cells can be realized under conditions of translational field. In this case, it should be admitted that the signals translated by NCT effectors are transmitted to acceptors differing from NK cells in their nature. This occurs not only during the contact with target cells and forced priming but also under other conditions. During maturation, activated NK cells acquire the potential sufficient for realization of cytotoxic and noncytotoxic regulatory effects. A high level of cooperativeness of cellular reactions in morphofunctional complex of regulation and control operating under the regimen of the interaction between NCT and IFN systems reflects (in this respect) natural evolutional prerequisite to performing EBR processes. This prerequisite is confirmed by the general scheme of organization and principles of realization of cellular activity.

Thus, structural scheme of EBR realized through the NCT system can be described by analogy with

functional organization of the IFN system as follows: translated (affecting and generated) molecular signal (cytokine), its receptor on the NK cell, effector of NCT itself, and target cell.

Study of NK Cells Function under EBR Regimen

The data mentioned above show the biological importance of endogenous translational processes in humans and higher animals. Methodology of EBR assuming the use of principally new approaches to analysis of intercellular interactions in the immune system requires experimental investigations and clinical testing. Study of this problem, its detail analysis, and elaboration of conceptual principles associated with EBR are important. The methodology of EBR is consistent with postulates and principles of general biology and immunology. This methodology is promising not only for theoretical biology but also for solving practical problems of immunoregulation and immunocorrection.

Let us consider the results of several clinical studies performed during IFN therapy. Treatment of patients with disseminated sclerosis with rIFN-α_2 increased the activity of NK cells only for a week. Cytotoxicity decreased below initial levels and was then restored after the end of therapy [60]. IFN-α used for therapy of patients with numerous warts induced the background for the cell response to IFN *in vitro* by increasing the activity of effectors. This was observed only during the first five weeks. The sensitivity was then lost [61]. The use of human leukocytic IFN for therapy of patients with melanoma stimulated NK cells during the first week of treatment. The initial level of cytotoxicity was then restored [48]. rIFN-α therapy of patients with lung cancer decreased the NK activity of lymphocytes after 2-4 weeks of treatment [63]. In patients with disseminated cancer of the large intestine, a short-term increase in NCT was followed by inhibition of effector functions registered during further treatment with IFN for three months [102]. Repeated injections of human rIFN-α and mouse rIFN-γ to mice decreased NK activity in all organs of animals [101].

In the context of immunopharmacology, the transitory and reversible nature of IFN effect mentioned above must be considered as the development of hyporeactivity typical of the immune deficiency diseases. In the context of EBR, by contrast, these facts must be interpreted as restoration of normal translational abilities of the system realized under conditions of certain restriction of the specialized activity. In the former case, the principles of therapy of patients indicate that it is necessary to discontinue this preparation and start immunostimulators of other nature. In the latter case, the principles of therapy suggest that it is necessary to continue supporting therapy with IFN. Moreover, the potency of IFN preparations used in clinical practice for treatment of viral infections, oncological diseases, and immune deficiency disorders is well-known.

Detailed analysis of the problem is necessary to consider EBR as an equivalent to the experience of long-standing experimental and clinical studies, and as the theory that can cause revision of the algorithms of evaluating patients' condition and the potency of immunotherapy. However, EBR is worth noticing because this theory can contribute to the proper choice of immunocorrection under conditions considered as ambiguous (using standard criteria).

Let us now turn to possibilities for study of the phenomenon and mechanisms providing EBR in cellular systems. It should be noted that instrumental difficulties associated with registration of translation of a regulatory signal may be probably obviated by a detailed comparative functional analysis of several elements of immunity involved (to a various extent) in EBR processes. This analysis would be promising because EBR recording can be only performed in the presence of cells (target cells) perceiving the translated signal in the experimental system (additionally to the presence of effectors). Therefore, functional complexes must be the basis for studies. In this case, cellular and humoral factors and mechanism that realize, regulate, and affect endogenous translational processes in humans and higher animals (and not a population of lymphoid cells involved in the reactions as an endogenous biological retranslator) will be the objects of these studies.

The logic of the studies assumes analysis of EBR processes at a certain level of tolerance. Physiological immunogenesis forms constantly renewing pool of circulating effector cells in the body. Precursors undergo continuous differentiation and enter consecutively various phases of maturation. These processes are not synchronized. In the present state, this is characterized (rather conventionally) only in model systems. Continuous differentiation of precursors recruit the circulation with heterogeneous (according to the maturation degree) cellular population. In this case, cell perceiving mediator regulatory signal can undergo further maturation or differential transformation into a specialized effector (over a short period of time). Because of activation of genes repressed in precursor and responsible for

the synthesis of a specific immunoactive compound, functional potential of the effector will be realized by generation of the "perceived" molecular signal. In this case, the cell perceiving the cytokine and the cell producing regulatory factor identical to this cytokine differ in the degrees of maturation. Therefore, these cells are not the same effector.

Now, these intercellular interrelations cannot be considered as an equivalent of EBR processes. The schemes shown in Fig. 1 are simplified, and variations of organization of translating complexes are hypothetical. They establish the functioning (under EBR regimen) of cellular effectors, which differentiation characteristics do not change in the reaction (from the beginning of action of immunoactive compound to production of its analogue). In future studies, the possibility of performing EBR during the passage of the effector cell through various phases of maturation must be either substantiated or completely excluded. For the time being, the constancy of translational functional complexes and certain invariability of their cellular composition (according to biological maturity) can be assumed.

It is important to understand that the development of immune response is associated with the presence of a considerable inductive constituent synchronizing functions of the cellular populations involved. Generalized activation of cells directed to realization of specialized potential, forming the pool of affecting effector cellular forms (over a short period of time), mobilizing helper cells, and initiating endogenous reinforcing mechanisms appears in the forefront. It seems likely that the processes that are not directly related to forced immunogenesis, provide intercellular interactions under physiological regimen and maintain immunoregulatory equilibrium in the complex of natural intercellular exchange (including EBR processes) must be delayed under these conditions. Therefore, extended immune response may be considered as process inhibiting physiological translation of regulatory signals of cytokines.

Thus, the EBR problem involves study of biological principles underlying the functioning of effector lymphocytes which combine the realization of cytotoxic and translational potentials. This suggests estimation (in the body) of the relationship between the mechanisms controlling realization of dif-ferentiated and genetically determined function by the cell and involvement of the cell into regulatory interactions considered in the context of EBR. It is necessary to reveal mechanisms of internal regulation of the NCT system responsible for translation of biological signals by this system under physiological regimen. The extent to which NK cells function autonomously in the immune reactions must be evaluated.

The problem of EBR should be analyzed in the context of processes associated with realization of the immune system ability for soft adaptation and rational compensation. This raises the question about the mechanisms of differentiation of signals of endogenous and exogenous inductors in peripheral compartments. Differentiation determines further selection and translation, which is adequate for functional needs and possibilities, of these signals to target cells. Analysis of EBR requires without studies of evolutionary determined principles of integration of the complex of immunobiological surveillance into open interactions with ecosystems and effects of environmental factors on biological retranslation performed by endogenous effector cells functioning in the branched network of cytokine regulation of immunogenesis (including the population of NK cells).

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REFERENCES

1. R. A. Burkharov, Yu. M. Zaretskaya, G. M. L'vitsyna, *et al.*, *Immunologiya*, No. 6, 84-86 (1984).
2. A. V. Karaulov, V. A. Dushkin, M. M. Intizarov, *et al.*, in: *Theoretical and Practical Problems of Gnotobiology* [in Russian], Moscow (1986), pp. 101-104.
3. S. A. Ketlinskii and N. M. Kalinina, *Immunologiya*, No. 3, 30-44 (1995).
4. I. V. Kondratenko, A. A. Yarilin, and L. N. Khakhalin, *Ibid.*, No. 1, 6-10 (1992).
5. V. A. Kuznetsov, *Ibid.*, No. 3, 8-13 (1992).
6. V. A. Kuznetsov, A. V. Ivshina, and Z. G. Kadagidze, *Ibid.*, No. 5, 27-30 (1988).
7. V. P. Kuznetsov, I. V. Korobko, V. Ya. Arion, *et al.*, *Vopr. Virusol.*, **32**, No. 5, 565-569 (1987).
8. A. Ya. Kul'berg, in: *Ekological Crisis:: Strategy of Survival* [in Russian], Moscow (1984).
9. E. Kuper, in: *Comparative Immunology* [in Russian], Moscow (1980).
10. A. S. Loginov, R. B. Gudkova, and V. B. Potapova, *Immunologiya*, No. 2, 11-14 (1992).
11. M. P. Potapnev, *Ibid.*, No. 4, 4-8 (1994).
12. Ya. I. Pukhova, I. A. Terskov, and L. D. Ponomarenko, *Dokl. Akad. Nauk SSSR*, **277**, No. 2, 509-512 (1984).
13. I. I. Slukvin, V. P. Chernyshov, A. V. Merkulova, *et al.*, *Immunologiya*, No. 5, 23-26 (1993).
14. A. M. Sorokin, S. B. Cheknev, and L. V. Koval'chuk, *Byull. Eksp. Biol. Med.*, **108**, No. 9, 322-325 (1989).
15. L. N. Fontalin, *Immunologiya*, No. 3, 5-20 (1988).
16. B. B. Fuks, A. G. Sterlina, and B. V. Nikonenko, in: *Immunological Aspeckts of Development at Biology* [in Russian], Moscow (1984), pp. 158-165.
17. S. B. Cheknev, *Immunologiya*, No. 6, 8-12 (1993).
18. S. B. Cheknev, *Ibid.*, No. 2, 9-17 (1994).
19. S. B. Cheknev, *Mechanisms Providing Regulatory Balance in the Complex of Natural Cytotoxic Reactions*. Abstr. MD Thesis [in Russian], Moscow, (1997).

20. S. B. Cheknev, A. M. Sorokin, and L. V. Koval'chuk, *Immunologiya*, No. 3, 48-52 (1989).
21. G. Z. Shubinskii and V. P. Lozovoi, *Ibid.*, No. 6, 17-22 (1984).
22. A. A. Yarilin, *Ibid.*, No. 4, 5-14 (1987).
23. M. Abbud-Filho, B. J. Ransil, V. E. Kelley, *et al.*, *Clin. Immunol. Immunopathol.*, **39**, No. 2, 264-276 (1986).
24. C. Agostini, R. Zambello, L. Trentin, *et al.*, *Am. Rev. Respir. Dis.*, **142**, No. 3, 516-522 (1990).
25. P. Allavena, G. Scala, J. Y. Djeu, *et al.*, *Cancer Immunol. Immunother.*, **19**, 121-126 (1985).
26. R. Aoki, T. Usuda, H. Miyakoshi, *et al.*, *Nat. Immun. Cell Growth Regul.*, **6**, 116-128 (1987).
27. G. C. Baron, N. G. Klimas, M. A. Fischl, and M. A. Fletcher, *Diagn. Clin. Immunol.*, **3**, No. 4, 197-204 (1985).
28. E. T. Bloom and J. T. Babbitt, *Cell. Immunol.*, **91**, No. 1, 21-32 (1985).
29. E. T. Bloom, J. T. Babbitt, and K. Kawakami, *J. Immunol.*, **137**, No. 1, 172-178 (1986).
30. V. Bocci, *Immunol. Today*, **6**, No. 1, 7-9 (1985).
31. V. Bocci, *Immunology*, **64**, 1-9 (1988).
32. M. Caligiuri, C. Murray, D. Buchwald, *et al.*, *J. Immunol.*, **139**, No. 10, 3306-3313 (1987).
33. S. Che and D. P. Huston, *Nat. Immun.*, **13**, No. 5, 258-269 (1994).
34. J. Chehimi, S. E. Starr, I. Frank, *et al.*, *J. Exp. Med.*, **175**, 789-796 (1992).
35. J. Chehimi, N. M. Valiante, A. D'Andrea, *et al.*, *Eur. J. Immunol.*, **23**, 1826-1830 (1993).
36. S. B. Cheknev, I. A. Tarkhanova, E. E. Babaeva, and A. J. Kulberg, *// Nat. Immun.*, **13**, No. 4, 233 (1994).
37. B. Combe, R. Pope, B. Darnell, and N. Talal, *Scand. J. Immunol.*, **20**, No. 6, 551-558 (1984).
38. E. L. Cooper, B. Rinkevich, G. Uhlenbruck, and P. Valembois, *Ibid.*, **35**, No. 3, 247-266 (1992).
39. F. Dianzani, *J. Interferon Res.*, Spec. Iss., May, 109-118 (1992).
40. A.-M. Dickinson, E. A. Jacobs, I. K. Williamson, *et al.*, *Clin. Immunol. Immunopathol.*, **49**, 83-90 (1988).
41. C. A. Dinarello, T. Ikejima, S. J. C. Warner, *et al.*, *J. Immunol.*, **139**, No. 6, 1902-1910 (1987).
42. J. Y. Djeu, N. Stocks, K. Zoon, *et al.*, *J. Exp. Med.*, **156**, 1222-1234, (1982).
43. L. Du Pasquier, in: *Fundamental Immunology*, 3rd ed., W. E. Paul (Ed.), New York (1993), pp. 199-233.
44. D. L. Evans, J. D. Folds, J. M. Petitto, *et al.*, *Arch. Gen. Psychiatry*, **49**, No. 5, 388-395 (1992).
45. B. C. Fellstrom and E. Larsson, *Immunol. Rev.*, **134**, 83-98 (1993).
46. M. Galli, A. Lazzarin, F. Saracco, *et al.*, *Clin. Immunol. Immunopathol.*, **50**, S166-S176 (1989).
47. P. R. Gibson and D. P. Jewell, *Austr. N. Z. J. Med.*, **15**, No. 1, Suppl. 1, 169 (1985).
48. S. H. Golub, P. D'Amore, and M. Rainey, *J. Natl. Cancer Inst.*, **68**, No. 5, 711-717 (1982).
49. C. E. Grossi and M. Ferrarini, in: *NK Cells and Other Natural Effector Cells*, R. B. Herberman (Ed.), New York (1982), pp. 1-8.
50. Z. Grossman and R. B. Herberman, *Cancer Res.*, **46**, 2651-2658 (1986).
51. J. E. Gumperz and P. Parham, *Monthly Nature*, **3**, No. 11, 58-61 (1995).
52. N. Hanna, *Biochim. Biophys. Acta*, **780**, No. 3, 213-226 (1985).
53. I. Hannet, F. Erkeller-Yuksel, P. Lydyard, *et al.*, *Immunol. Today*, **13**, No. 6, 215-218 (1992).
54. M. Hansson, M. Petersson, G. C. Koo, *et al.*, *Eur. J. Immunol.*, **18**, 485-488 (1988).
55. R. B. Herberman, *Invest. Cell. Pathol.*, **1**, 227-248 (1978).
56. R. B. Herberman, *Mol. Immunol.*, **19**, No. 10, 1313-1321 (1982).
57. R. B. Herberman and H. T. Holden, *J. Natl. Cancer Inst.*, **62**, No. 3, 441-445 (1979).
58. R. B. Herberman and J. R. Ortaldo, *Science*, **214**, 24-30 (1981).
59. T. Hercend and R. E. Schmidt, *Immunol. Today*, **9**, No. 10, 291-293 (1988).
60. R. L. Hirsch and K. P. Johnson, *Clin. Immunol. Immunopathol.*, **37**, No. 2, 236-244 (1985).
61. M. Ho, L. T. White, H. W. Haverkos, *et al.*, *J. Infect. Dis.*, **144**, No. 6, 605 (1981).
62. P. G. Hogan., A. J. Hapel, and W. F. Doe, *J. Immunol.*, **135**, No. 3, 1731-1738 (1985).
63. P. Hokland, M. Hokland, B. K. Olesen, and P. Ernst, *Antiviral Res.*, Spec. Abstr. Iss., **4**, No. 3, 73 (1984).
64. L. A. Holmberg, B. A. Miller, and K. A. Ault, *J. Immunol.*, **133**, No. 6, 2933-2939 (1984).
65. A. Isaacs and D. C. Burke, *Nature*, **182**, 1073-1074 (1958).
66. K. Karre, M. Hansson, and R. Kiessling, *Immunol. Today*, **12**, No. 10, 343-345 (1991).
67. T. Kasahara, J. Y. Djeu, S. F. Dougherty, and J. J. Oppenheim, *J. Immunol*, **131**, No. 5, 2379-2385 (1983).
68. P. Katz, S. R. Mitchell, T. R. Cupps, *et al.*, *Cell. Immunol.*, **119**, 130-142 (1989).
69. K. Kawakami and E. T. Bloom, *Ibid.*, **116**, 163-171 (1988).
70. K. L. Kelly, E. L. Cooper, and D. A. Raftos, *J. Exp. Zool.*, **262**, 202-208 (1992).
71. N. U.-D. Khan, K. A. F. Pulford, M. A. Farquhanson, *et al.*, *Immunology*, **66**, No. 2, 201-206 (1989).
72. R. Kiessling, M. Hansson, and A. Gronberg, *Progr. Immunol.*, **5**, 1181-1194 (1984).
73. E. Kott, T. Hahn, M. Huberman, *et al.*, *Q. J. Med.*, 76, No. **281**, 951-960 (1990).
74. A. J. Kulberg, E. E. Babaeva, I. A. Tarkhanova, *et al.*, *Biotechnology*, **9**, No. 2, 123 (1994).
75. A. J. Kulberg, F. I. Yershov, I. A. Tarkhanova, *et al.*, *J. Interferon Res.*, **13**, Suppl. 1, 108 (1993).
76. L. L. Lanier, H. Spits, and J. H. Phillips, *Immunol. Today*, **13**, No. 10, 392-395 (1992).
77. H. B. Levy, C. E. Buckler, and S. Baron, *Science*, **152**, 1274-1276 (1966).
78. C. E. Lewis, A. L. Ramshaw, J. Lorenzen, and J. O'D. McGree, *Cell. Immunol.*, **132**, No. 1, 158-167 (1991).
79. H. Li, R. Schwinzer, M. Baccarini, and M.-L. Lohmann-Matthes, *J. Exp. Med.*, **169**, No. 3, 973-986 (1989).
80. J. L. Marx, *Science*, **210**, 624-626 (1980).
81. N. Minato, T. Amagai, J. Yodoi, *et al.*, *J. Exp. Med.*, **162**, No. 4, 1161-1181 (1985).
82. D. Montagna, R. Maccario, A. G. Ugazio, *et al.*, *Clin. Exp. Immunol.*, **71**, No. 1, 177-181 (1988).
83. M. P. N. Nair, S. A. Schwartz, and M. Menon, *Cell. Immunol.*, **94**, No. 1, 159-171 (1985).
84. B. Naume, M. K. Gately, B. B. Desai, *et al.*, *Cytokine*, 5, 38-46 (1993).
85. B. Naume, M. Gately, and T. Espevik, *J. Immunol.*, **148**, No. 8, 2429-2436 (1992).
86. M. Ono, N. Tanaka, and K. Orita, *Acta Med. Okayama.*, **38**, No. 2, 207-213 (1984).
87. R. Pardi, J. R. Bender, C. Dettori, *et al.*, *J. Immunol.*, **143**, No. 10, 3157-3166 (1989).
88. M. Provinciali, G. Di Stefano, W. Raffaeli, *et al.*, *Int. J. Neurosci.* **59**, 127-133 (1991).
89. H. Rabinowich, Y. Goses, T. Reshef, and A. Klajman, *Mech. Ageing Dev.*, **32**, No. 2-3, 3-226 (1985).
90. D. M. Radomska, A. Osiecka, and J. Malejczyk, *Immunol. Cell Biol.*, **67**, No. 4, 209-213 (1989).
91. C. P. Robles and S. B. Pollack, *Nat. Immun. Cell Growth Regul.*, **5**, No. 2, 64-74 (1986).

92. B. Rolstad, S. Fossum, H. Bazin, *et al.*, *Immunology*, **54**, No. 1, 127-138 (1985).
93. E. Saksela, T. Timonen, and K. Cantell, *Scand. J. Immunol.*, **10**, 257-266 (1979).
94. C. A. Savary and E. Lotzova, in: *Immunobiology of Natural Killer Cells*, E. Lotzova and R. B. Herberman (Eds.), Boca Raton, CRC (1986), **1**, pp. 45-61.
95. S. A. Serrate, R. S. Schulof, L. Leondaridis, *et al.*, *J. Immunol.* **139**, No. 7, 2338-2343 (1987).
96. S. Shimada and S. Katz, *Arch. Pathol. Lab. Med.*, **112**, 231-234 (1988).
97. J. A. Smith, *J. Leukoc. Biol.*, **56**, No. 6, 672-686 (1994).
98. K. Soderhall, A. Wingren, M.W. Johansson, and K. Bertheussen, *Cell. Immunol.*, **94**, No. 2, 326-332 (1985).
99. H. Strander, *Production of Interferon by Suspended Human Leukocytes*, Stockholm, 1971.
100. R. Suzuki, S. Suzuki, N. Ebina, and K. Kumagai, *J. Immunol.*, **134**, No. 4, 2139-2148 (1985).
101. J. E. Talmadge, R. B. Herberman, M. A. Chirigos, *et al.*, *Ibid.*, **135**, No. 4, 2483-2491.
102. B. Tank, A. M. Bijma, R. L. Marquet, *et al.*, *Oncology*, **42**, 157-163 (1985).
103. S. Targan and F. Dorey, *J. Immunol.*, **124**, No. 5, 2157-2161 (1980).
104. J. Tarkkanen and E. Saksela, *Scand. J. Immunol.*, **15**, 149-157 (1982).
105. Z. Toossi, M. R. Paris, S. F. Purvis, and J. J. Ellner, *Cell. Immunol.*, **118**, No. 2, 413-424 (1989).
106. J. M. Tsuji, S. B. Pollack, *Nat. Immun.*, **14**, No. 1, 44-56 (1995).
107. P. Valenbois, P. Roch, D. Boiledieu, in: *The Reticuloendothelial System: A Comprehensive Treatise*, N.Cohen and M.M. Sigel (Eds.), New York (1982), **3**, pp. 89-139.
108. M. Vitale, L. Zamal, L. M. Neri *et al.*, *Cell. Immunol.*, **145**, No. 1, 1-10 (1992).
109. B. Von Gaudecker, U. Pfingsten, and H.-K. Muller-Hermelink, *Cell Tissue Res.*, **238**, No. 1, 135-143 (1984).
110. S. J. C. Warner, K. R. Auger, and P. Libby, *J. Immunol.*, **139**, No. 6, 1911-1917 (1987).
111. H. S. Warren, B. F. Kinnear, and L. J. Skipsey, *Immunol. Cell Biol.*, **71**, No. 2, 87-97 (1993).
112. P. M. Waterfall, L. S. Rayfield, and L. Brent, *Transplant. Proc.*, **17**, No. 1, Book 2, 1140-1142 (1985).
113. T. L. Whiteside and R. B. Herberman, *Clin. Immunol. Immunopathol.*, **53**, 1-23 (1989).
114. M. Wittke and L. Renwrantz, *J. Invertebr. Pathol.*, **43**, No. 2, 248-253 (1984).
115. H. Young and J. Ortaldo, *J. Immunol.*, **139**, No. 3, 724-727 (1987).
116. F. Yu, Y. Itoyama, K. Fujihara, and I. Goto, *J. Neuroimmunol.*, **33**, No. 2, 121-128 (1991).
117. B. Zweiman and R. P. Lisak, *Hum. Pathol.*, **17**, No. 3, 234-245 (1986).