Immunocytotherapy. A New Trend in Fetal Tissue Transplantation

V. I. Kulakov, G. T. Sukhikh, E. M. Molnar, V. V. Malaitsev, I. M. Bogdanova, and M. Yu. Sutyrin

UDC 618.33-018-089.843

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 117, № 4, pp. 412-415, April, 1994
Original article submitted March 10, 1994

The clinical applications of theoretical immunology are not numerous. The authors demonstrate present-day immunocytotherapeutic approaches to fetal tissue transplantation widely used in medicine. Results of therapy by injections of lymphokine-activated killer cells in combination with interleukin-2, T-cell vaccines, and genetically transformed T cells are presented.

Key Words: *immunocytotherapy; fetal tissue transplantation*

Progress in fundamental immunology is one of the most impressive achievements of the twentieth century. The very first results attained at the end of the nineteenth and the beginning of the present century were met with enthusiasm by contemporaries. Discoveries in immunology not only promoted a broader outlook on the structure of all living things, but to a great extent were conducive to creating conditions for introducing the results of new discoveries into practical medicine. Recent years, however, have brought about an evident dissonance between the burgeoning progress in basic research and the rather modest contribution of this science to present-day practical medicine. The seeming inability of immunologists to solve pressing problems of controlling AIDS, Legionaires' disease, chronic fatigue syndrome, etc., is dismaying. Problems in the therapy of many other prevalent infections are also far from being solved. The expected progress in immunooncology is still to be attained. Many difficulties remain in connection with transplantations of organs and tissues.

In this paper we describe some new results in fundamental and applied immunology which for the most part are little known to the reader but are indicative of a promising contribution of this science to practical medicine.

We believe that progress may be achieved by using intact and genetically modified immunocompetent cells in the therapy of oncologic, autoimmune, and some viral diseases. As far as practical medicine is concerned, we hope to make our own contribution to the development of immunocytogenotherapy. The intensive research in the field of fetal tissue transplantation that is being carried out at the International Institute of Biological Medicine gives us grounds for optimism.

Immunocytotherapy may become one of the most fruitful trends in fetal tissue transplantation. The first practical results in this field were attained by an American scientist, S. Rosenberg, who treated a number of oncologic diseases by administering to patients cytotoxic effectors of antitumor immunity, lymphokine-activated killer (LAK) cells, in combination with recombinant interleukin-2 [14,16,17]. Peripheral blood lymphocytes were *in vitro* stimulated with interleukin-2, and then mature LAK were intravenously injected to the same patient. A simultaneous administration of exogenous interleukin-2 helped maintain the functional status of the cytotoxic effectors. The best results were obtained in the treatment of renal cancer, mela-

International Institute of Biological Medicine, Moscow

0007-4888/94/0004-0411$12.50

nomas, colon cancer, and non-Hodgkin's lymphomas. Such an approach, however, involves the need to inject recombinant interleukin-2 in doses which may have a toxic effect on the body. Some patients have developed shifts in the psychoemotional sphere and toxic involvement of the kidneys. Still, Rosenberg's experiments lent a strong boost to research in immunocytotherapy [15,18]. Its use in practical oncology aroused the greatest expectations. Animal experiments demonstrated the efficacy of using so-called tumor-associated lymphocytes. It was shown that among T lymphocytes infiltrating malignant tissue there are cells potentially capable of tumor destruction. These cells may be isolated from the body and, after *in vitro* culturing under conditions providing for their expansion, replaced in the tumor host organism. These T lymphocytes, specific to this very tumor, upon becoming redistributed in the body, migrate into the tumor growth zone and accumulate there, later to eliminate the malignant cells, resulting in tumor regression [15,21].

Such treatment can be effective only if a favorable quantitative balance is created between the injected effector T lymphocytes and the cells providing for their microenvironment in tumor tissue (other immunocompetent and helper cells as well as tumor cells proper).

Attempts to change this balance in favor of the lymphocytes causing tumor destruction have not always been successful. But then a significant extra component was introduced into the studies. To improve the cytolytic function of tumor-associated lymphocytes, the tumor necrosis factor (TNF) gene was transfected into these cells. After transfectant cell transfer to tumor-bearing animals, these cells migrated into the tumor tissue and secured a high local concentration of TNF, this leading to the death of TNF-sensitive tumor cells but not causing any side effects due to the action of this pleiotropic cytokine on intact tissues of the body. This new approach has been clinically assayed in 4 patients with melanomas and the first encouraging results have been obtained [15]. The goal is to enhance the antitumor activity of tumor-associated T lymphocytes by transfecting into these cells the genes of γ-interferon, interleukins-2 and -6, as well as of chimeric T-cell receptors. It was shown that cytokine gene transfection in tumor cells is conducive to the improvement of their immunogenicity for host cytotoxic cells [15]. Transfection of interleukins-2 and -4, TNF, γ-interferon, and granulocytic-macrophagal colony-stimulating factor genes in tumor cells to enhance their immunogenicity is proposed. Tumor immunogenicity may be improved by transfection of the class 1 major histocompatibility complex gene [15].

Hence, a qualitatively new direction of fetal tissue transplantation has emerged at the interface between molecular biology and cell immunology, a direction which may lend impetus to the development of modern methods of treatment of not only tumors but, possibly, of a wide range of etiologically different diseases as well.

Another promising approach consists in the construction of so-called T-cell vaccines [12,13]. The efficacy of T-cell vaccination as a specific prophylactic and therapeutic procedure has been proven in animals with experimentally induced autoimmune diseases. Immunization with attenuated T cells specific for an autoantigen (T-cell vaccination) ensures protection against the induction of an autoimmune process or prevents its development according to an antigen-specific mechanism [3,5,7,11,19]. This was persuasively demonstrated in a case of development of adjuvant arthritis in which *Mycobacterium tuberculosis* caused an autoimmune process [1,8]. The efficacy of T-cell vaccination is due to activation or modulation in the system of antiidiotypic and other antigen-specific and nonspecific regulatory cells and factors. These disturbances in the immune system eventually lead to reduction of the activity of effectors responsible for the formation of the autoimmune status. Thus, in disseminated sclerosis T lymphocytes expressing the Vβ17 segment of T-cell receptors of the family V have a very high incidence in T-cell lines specific to epitopes of myelin basic protein [22]. Vβ12.1+T cells predominate in the brain tissue of patients with disseminated sclerosis. Mono- and oligoclonal activation of autoreactive T lymphocytes has been described in autoimmune hypothyroidism and rheumatoid arthritis [12,20,22].

In accordance with current notions of autoimmunity and its possible relationship with infection by foreign pathogens, effector T cells directly implicated in the development of autoimmune diseases are represented in normal subjects; but their activity is modulated and in health suppressed at the expense of systemic interactions of regulatory T cells. Because of mimicry between molecular components of microorganisms and antigens proper, an autoimmune process may be triggered specifically by foreign pathogens which cause a disturbance in the immunoregulatory network with activation predominating over suppression.

In T-cell vaccination autologous effector T cells reinjected after *in vitro* activation and attenuation change the regulatory balance toward the suppressor component of immunity.

The first successful results of treating some human autoimmune diseases with T-cell vaccines have been described [8,10]. In 6 patients with disseminated sclerosis an antiidiotypic inhibitory response was observed after T-cell vaccination with T-lymphocyte clones specific to myelin basic protein. Van Laar (1993) described the effective use of T-cell vaccines in therapy of patients who had been suffering from rheumatoid arthritis for an average of 12.8 years. A reduction of autoimmune process activity was observed, being most marked in patients with a shorter history of the disease [2].

Methods of T-cell vaccination for type I diabetes mellitus are being developed in animal experiments. T-lymphocyte autoaggression against Langerhans islet β cells underlies the development of this disease in NOD mice. At early stages of spontaneous development autoantigen-specific cytolysis of β cells is mediated by Vβ8+T lymphocytes. T lymphocytes recognizing Vβ8 at the surface of autoaggressive T cells were obtained *in vitro*. After transfer of these cells to NOD mice a clear-cut reduction of the disease activity due to the suppression of autoaggressive T cells function was observed [9].

As mentioned above, a limited number of T-lymphocyte antigen-specific clones are involved in the pathological process at early stages of many other autoimmune diseases of man and animals.

The oligoclonal expansion of autoimmune T lymphocytes with predominant expression of individual T cell receptor variable genes associated with this disease permits us to hope that multipurpose broad-spectrum T-cell vaccines will be developed [4]. This is connected with the possibility of using as vaccines not only autologous, but allogenic T cells as well, because an idiotype-antiidiotypic pattern of interactions between vaccinal T cells and recipient T lymphocytes is postulated.

At present the use of T-cell vaccines to combat the twentieth century-plague, AIDS, is being investigated [2]. Autologous T cells specifically proliferating on the viral coat glycoprotein, gp120, and (or) manifesting HLA-restricted *in vitro* cytotoxicity against CD4+ T cells are suggested as a vaccine. This vaccine should first of all reduce autoimmunopathy toward gp120 or gp120 cross-reactive antigens. The reduced immunoprotective effect induced by the vaccination should not seriously impact treatment quality, because autoimmunopathy, not viral cytopathy, is the key mechanism of AIDS development. Besides, the suggested vaccination method should help suppress the production of neutralizing viral antibodies in the presence of intensified function of virus-specific cytotoxic T cells. This aspect is of paramount importance, for the efficacy of antiviral immunity is determined mainly by T-cell function.

Hence, immunocytotherapy may be an effective method for the treatment of oncologic and autoimmune diseases. The efficacy of immunocytotherapy may be markedly increased by gene transfection in immunocompetent cells. This applies equally to the transfer of viable functionally sound as well as attenuated "vaccinal" T lymphocytes [6].

In the first case cell modification may consist in the preparation of transfectants characterized by constitutive or induced cytokine production. We illustrated this assumption above when describing the tumoricidal effect of tumor-associated T lymphocytes in which the TNF gene was transfected. In such a case all lymphocytes affine to the tumor, whatever their initial appurtenance to a particular T-cell subpopulation, became cytotoxic effectors, and local high TNF production ensured a high efficacy of tumor destruction. This circumstance is particularly important if we bear in mind that in cancer patients, especially at the late stages of the disease, excessive endogenous TNF production causes the development of cachexia (a synonym for TNF is cachectin), but at the same time TNF, because of an insufficient local concentration in the tumor, not only does not destroy it but even fails to prevent generalization of the pathological process.

Another example is the use of transfectant cells producing transforming growth β factor (TGF-β) for wound healing.

It was shown in rat experiments that fibroblasts transfected with TGF-β gene, when applied to a wound surface, were conducive to a more rapid regeneration of the skin [15].

In principle, it is possible to use lymphocytes transfected with TGF-β to intensify repair processes in organs and tissues. The possibility of ensuring strictly addressed label due to the capacity of memory T lymphocytes to selectively penetrate into involved tissue is an advantage of such an approach. These cells, owing to a selective pattern of interactions with vascular endothelium of various tissues, migrate into the so-called "tertiary lymphoid organs," that is, into organ and tissue structures not belonging to the lymphoid system, provided that an inflammatory process develops in them. The selective penetration of individual memory T cells is not connected with their antigenic specificity but is due to the presence of respective tissue endotheliocytes on the cell surface

of adhesion molecules interacting with the counter-receptors. To solve the problem of T cell transfer to a specified compartment of the body and to secure production of a necessary factor, double transfectants may be constructed that simultaneously express the adhesion molecule (as an address marker) and the relevant factor (e.g., TGF-β).

Such an approach is in principle possible in the creation of T-cell vaccines where adhesion molecule gene transfection increases the possibility of cellular vaccine penetration into the right compartment. Transfectants carrying an address label and a certain variant of T-cell receptor may be designed if necessary. In the latter case any lymphocyte, whatever its migration capacity and type of antigen-recognizing structure, may be an object of transfection.

In fact, the potentialities of the new approach, immunocytogenetic therapy, depend on the scope of our knowledge about the mechanisms of individual pathological processes; as far as the development and technical preparation of cellular "drugs" is concerned, the sky is the limit.

Within a relatively short period of development fetal tissue transplantation has undergone significant transformation: from an empirical initial stage, when its progress depended solely on the practical experience and intuition of clinicians, to the stage based on the latest findings in molecular and cell biology: the goal-directed construction of genetically modified structures (transfectant cells) that also correct processes of intercellular relations in the body.

REFERENCES

1. H. Atlan and I. R. Cohen, in: *Theoretical Immunol. and Exp. Insights into Immunology* (1992), pp.379-395.
2. H. Atlan *et al., Immunol. Today,* **14**, 200-202 (1993).
3. E. Beraud, *Ann. New York Acad. Sci.,* **636**, 124-134 (1991).
4. S. W. Brostoff and M. D. Howell, *Clin. Immunol. Immunopathol.,* **62**, 1-7 (1992).
5. I. R. Cohen, *Cold Spring Harb. Symp. Quant. Biol.,* **54**, 879-884 (1989).
6. I. R. Cohen, *J. Intern. Med.,* **230**, 471-477 (1991).
7. I. R. Cohen and H. Atlan, *J. Autoimmun.,* **2**, 613-625 (1989).
8. I. R. Cohen and D. B. Young, *Immunol. Today,* **12**, 105-110 (1991).
9. B. Formby and T. Shao, *Ann. Clin. Lab. Sci.,* **23**, 137-147 (1993).
10. D. A. Hafler *et al., Clin. Immunol. Immunopathol.,* **62**, 307-313 (1992).
11. D. Klatzman and L. Montagnier, *Nature,* **319**, 10-11 (1986).
12. A. M. Mittenburg *et al., Scand. J. Immunol.,* **31**, 121 (1990).
13. P. A. H. Moss *et al., Ann. Rev. Immunol.,* **10**, 71-96 (1992).
14. S. A. Rosenberg, *J. Biol. Response Modif.,* **3**, 501-511 (1984).
15. S. A. Rosenberg, *Cancer Res.,* Suppl. 51, 5074s-5079s (1991).
16. S. A. Rosenberg *et al., New Engl. J. Med.,* **313**, 1485-1492 (1985).
17. S. A. Rosenberg *et al., Ibid.,* **319**, 1676-1680 (1988).
18. S. A. Rosenberg *et al., Ibid.,* **323**, 570-578 (1990).
19. C. Roubaty *et al., J. Immunol.,* **144**, 2167-2172 (1990).
20. I. Stamenkovic *et al., Proc. Nat. Acad. Sci. USA,* **85**, 1179 (1988).
21. S. L. Topalian *et al., J. Immunol. Meth.,* **102**, 127-141 (1987).
22. K. W. Wucherpfennig *et al., Science,* **248**, 1016-1019 (1990).