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MODERN APPROACHES OF MOLECULAR GENETICS AND IMMUNE BARRIERS OF GENE THERAPY

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Abstract

The article provides an overview of local publications in Russian Federation from 2020-2025 devoted to the immunological aspects of gene therapy.

The aim of the work was to systematize the data of the Russian literature on the key immune barriers limiting the effectiveness of viral vectors and genome editing technologies, as well as to summarize the approaches discussed in publications to overcome them.

It has been shown that the most significant obstacles to gene therapy remain pre-existing and induced neutralizing antibodies to viral capsids, congenital inflammatory reactions, T-cell deletion of the transduced cells, as well as the immunogenicity of the transgenic product.

For systems based on adeno-associated viruses, the impossibility of safe re-administration and the need to reduce the therapeutic dose by increasing the targeting of delivery are of particular importance.

For genome editing technologies, immunological risks are considered inextricably linked to DNA damage, the immunogenicity of bacterial nucleases, and genomic safety issues.

In the oncological field, the immune response is twofold: it can limit the duration of the virus's persistence, but at the same time participate in the formation of an antitumor effect. Preliminary immunological screening, modification of capsids and regulatory elements, selection of the optimal route of administration,

strategies and short-term controlled immunomodulation are discussed as the main ways to overcome barriers in the Russian literature.

Keywords: gene therapy, immunogenicity, AAV, viral vectors, genome editing, oncolytic viruses, immune response, overcoming barriers.

INTRODUCTION

In recent years, gene therapy in the Russian literature has finally ceased to be considered only as a promising experimental technology and is increasingly described as an independent platform of drug action combining substitution, regulatory and antitumor approaches [4, 10]. Along with the expansion of the range of therapeutic tasks, the scientific focus has also shifted: if early discussions focused mainly on the very possibility of delivering a genetic construct into a cell, modern reviews pay primary attention to the quality of the vector's interaction with the patient's immune system, which determines both the severity of the effect and its duration.

It is the immunological limitations in the works of 2020-2025 that are most often identified as the most stable barrier to the clinical implementation of gene therapy. [1, 5, 8, 9]. The Russian authors emphasize that the immune response in this case cannot be reduced to just one mechanism for neutralizing the viral carrier. We are talking about a cascade of interrelated processes, including recognition of foreign proteins by innate immunity, activation of a humoral response, cell-mediated elimination of transduced cells, as well as reactions to a newly synthesized therapeutic protein. Therefore, even a technologically advanced design may not be clinically effective enough if its use is accompanied by immune rejection or severe inflammation.

Russian publications analyze vectors based on adeno-associated viruses in the most detail, which are characterized by a combination of a relatively favorable safety profile and high sensitivity to immunological factors. [5, 6, 7, 9]. Before the start of therapy, some patients already have antibodies to natural AAV serotypes, and after the introduction of the recombinant vector, a de novo humoral response is rapidly formed, making it difficult to achieve the target bioavailability and virtually eliminating safe re-administration. Additional complexity is created by cellular mechanisms for the removal of transduced cells and the possibility of a lack of immunological tolerance to the protein being restored.

A special profile of immunological risks is characteristic of genome editing technologies and oncolytic virotherapy [2, 3, 8]. In the first case, not only reactions to elements of the editing system, including bacterial nucleases, are discussed, but also the relationship of immune inflammation with DNA damage and off-target effects. In the second case, the antiviral response acquires a dual role: it can prematurely limit the replication of the oncolytic agent, but at the same time participates in the launch of antitumor immunity. This difference in mechanisms makes the problem of immunological barriers heterogeneous and requires a platform-specific analysis.

For Russian authors, immunological issues are also important because they combine fundamental and applied levels of analysis [1, 4, 8]. The same immune phenomenon simultaneously affects the pharmacokinetics of the vector, the safety of the drug, the design of preclinical studies, and the choice of clinical strategy. Therefore, the discussion of barriers in modern Russian reviews goes far beyond a narrow description of adverse reactions and is considered as a central condition for the rational design of gene therapy platforms.

The purpose of this review article is to analyze Russian publications from 2020-2025 devoted to the immunological aspects of gene therapy, highlighting the main barriers to its effectiveness and systematizing ways to overcome them, discussed in modern Russian literature.

MATERIALS AND METHODS OF RESEARCH

The work was performed as a review and analytical study of the literature without conducting its own experiment, clinical observation or other practical part. The publications of Russian authors are used as an information base. The search was conducted using combinations of keywords "gene therapy", "immunogenicity", "AAV", "viral vectors", "genome editing", "oncolytic viruses", "neutralizing antibodies", "safety".
The inclusion criteria were: the affiliation of the publication to the Russian scientific space, the publication period from 2020 to 2025, the review or analytical nature of the work, the availability of data on the immune response to the vector, transgene or editing system, as well as a discussion of risks and ways to reduce them. Materials devoted exclusively to molecular aspects without immunological interpretation, foreign sources and publications duplicating already selected reviews were excluded from further analysis. The 10 most relevant domestic sources were selected for the final analysis.

RESULTS AND DISCUSSION

An analysis of the Russian literature shows that immunological barriers in gene therapy are multilayered and arise at different stages of the treatment process — from the moment of systemic vector administration to long-term expression of the therapeutic gene [1, 5, 9]. At the initial stage, the mechanisms of innate recognition and the initial immune memory to viral components are crucial. At subsequent stages, the clinical outcome is determined not only by the delivery of the genetic construct, but also by whether the transduced cell can avoid immune deletion and ensure stable production of the target protein.
The issue of immunogenicity of adeno-associated viral vectors is the most developed in domestic reviews. Despite their reputation as a relatively safe platform, AAVs are not immunologically "invisible" to the body [5, 7, 9]. The publications emphasize that previous human contact with natural serotypes leads to the formation of neutralizing antibodies even before the start of treatment. This situation is of fundamental importance for in vivo therapy, since even a low antibody titer can reduce the proportion of vector delivered to tissues and increase dosage requirements.
The problem of pre-existing humoral immunity is especially significant for systemic administration of high doses of AAV, when the therapeutic effect depends on whether a significant portion of the viral particles can reach the target organ without being rapidly inactivated in the bloodstream [5, 6, 9]. The Russian authors attribute to this the need for mandatory immunological screening of patients before therapy, since a universal treatment regimen for all serological variants of the population in such conditions turns out to be unrealistic. An additional complication is created by the cross-reactivity of antibodies to different serotypes, which limits the possibilities of simply switching from one capsid to another.
Even in the absence of initial antibodies, an induced humoral response develops after the initial administration of the recombinant vector, which makes the issue of repeated administration one of the most difficult in modern gene therapy. [5, 6, 7, 9]. This feature has not only technical, but also strategic importance. If the duration of expression decreases, and repeated delivery of the same vector carries a high risk of neutralization, then the developer is forced to design the therapy in advance as effective as possible already at the first administration. Hence, there is an increasing interest in platforms with increased affinity, local delivery routes, and constructs that provide sufficient effect with fewer viral particles.
The humoral response, however, does not exhaust the totality of immunological limitations. A number of Russian studies have noted the importance of cellular immunity, which is able to recognize capsid antigens or transgene expression products on the surface of transduced cells [1, 4, 6]. This is especially important in situations where, for one reason or another, the therapeutic protein is perceived by the immune system as partially foreign. In hereditary diseases with complete or almost complete loss of its own protein, restored expression may not be accompanied by full-fledged tolerance, and then the very purpose of therapy becomes a trigger for immune conflict.
Thus, immunogenicity in gene therapy is related not only to the carrier, but also to the biological features of the therapeutic design. The Russian authors draw attention to the fact that the choice of promoter,

expression level, and tissue specificity plays no less a role than the properties of the capsid. [1, 4, 7, 10]. Excessive, spatially unlimited expression of the transgene increases the likelihood of its immunological presentation in undesirable cell populations. On the contrary, the use of targeted regulatory elements makes it possible to simultaneously reduce the therapeutic dose and narrow the area of immune interaction, making treatment more manageable.

A separate set of problems is related to the dose-dependent nature of immune and inflammatory reactions. A number of Russian reviews emphasize that increasing the dose of the viral vector is often considered as a direct way to enhance the therapeutic effect, but in practice this approach quickly runs into safety limitations [1, 5, 7]. The greater the vector load, the higher the probability of a systemic inflammatory reaction, the more noticeable the antigenic exposure of capsid proteins and the stronger pressure on the immune system. Therefore, the modern logic of development is shifting from simply increasing doses to increasing the targeted delivery and functional efficiency of each injected particle.

In publications devoted to hereditary diseases, this thesis is given special importance. When it is necessary to transduce large amounts of tissue, for example, skeletal muscles, an attempt to compensate for insufficient efficacy only by increasing the dosage inevitably exacerbates immunological and toxicological risks [5, 6]. Hence, there is an interest in engineering modification of capsids, in choosing serotypes with more pronounced organ tropicity, as well as in routes of administration that reduce systemic exposure. In fact, the task of overcoming the immune barrier is gradually turning into a task of precise bioengineering the platform.

The route of administration of the gene therapy drug is also essential. The Russian authors emphasize that systemic delivery, although it provides a wide coverage of tissues, is more often associated with maximum contact of the vector with the immune system of the blood and reticuloendothelial bed [5, 7, 9]. Local and regional methods of administration are not a universal solution, but in many cases, they can reduce the antigenic load, reduce the proportion of neutralized particles and This increases the efficiency-to-risk ratio. Therefore, the immunological barrier is determined not only by the properties of the construct, but also by the biological route along which it reaches the target cell.

A specific dimension of immunological problems is emerging in the field of genome editing. Russian authors consider such technologies not only through the prism of the effectiveness of mutation correction, but also through the risks associated with the immunogenicity of bacterial nucleases, the induction of double-stranded DNA breaks and the activation of stress cellular responses [1, 8]. Here, the immunological component is closely intertwined with genomic safety: DNA damage can be accompanied by inflammatory signaling, and immune recognition of proteins in the editing system can shorten their action time or enhance the damaging effect. Therefore, reducing immunogenicity in such systems is inseparable from controlling the duration of expression and minimizing off-target events.

The issue of preclinical assessment of immunological risks deserves special attention. Domestic safety reviews emphasize that immunogenicity should not be considered as a secondary indicator detected after demonstrating therapeutic efficacy [1, 4]. On the contrary, the assessment of the likelihood of inflammatory reactions, antibody formation, cellular cytotoxicity, and immunogenicity of a therapeutic protein should be integrated into the development architecture at an early stage. This approach allows us to filter out constructs with a deliberately unfavorable profile and proceed to a clinical discussion of not only "working" but also immunologically acceptable solutions.

That is why ex vivo strategies look particularly promising for genomic editing, in which cell modification is carried out outside the body, followed by controlled return of the therapeutic product to the patient [4, 8, 10]. This approach does not completely eliminate immunological risks, but it can significantly reduce the systemic effects of editorial proteins and viral carriers on the body. In addition, it creates opportunities for pre-selection of successfully modified cells, which reduces the need for excessive exposure to the vector and reduces the likelihood of a pronounced immune response in vivo.

Oncolytic virotherapy occupies a special place in the Russian literature, where the immune response is assessed not as an exclusively undesirable phenomenon, but as one of the carriers of the therapeutic effect [2, 3]. Oncolytic viruses destroy tumor cells directly, but at the same time create conditions for the release of tumor antigens, local inflammation, and activation of antitumor immunity. In this case, the barrier and the therapeutic resource partially coincide. An overly rapid antiviral response can shorten the duration of viral replication and spread in a tumor, but it is also impractical to completely suppress the immune response in such a model, since this may weaken the systemic antitumor result.

This leads to an important methodological conclusion: there is no universal scheme of immunological support for all areas of gene therapy. [2, 3, 5, 8]. For substitution therapy in hereditary diseases, the priority is to prevent the neutralization of the vector and preserve the long-term expression of the transgene. For genome editing systems, short duration, controllability, and genomic safety of exposure come to the fore. Oncolytic platforms, on the contrary, require fine-tuning the balance between preserving the replicative potential of the virus and stimulating antitumor immune activity.

The ways to overcome immunological barriers discussed by the Russian authors can be reduced to several interrelated areas, which, however, do not form a simple set of techniques. [1, 5, 7, 9, 10]. First, personalized immunological screening before starting treatment is of key importance, including the assessment of neutralizing antibodies and, if necessary, the choice of an alternative vector platform. Secondly, much attention is paid to the engineering of capsids and targeted regulatory elements that reduce the required dose and redistribute the vector in favor of the target tissue. Thirdly, the role of local and regional delivery methods is growing, which limit the systemic contact of the vector with the immune system.

The Russian literature also focuses on the temporal dimension of the immune problem [1, 5, 10]. Even a successful initial administration does not guarantee a stable long-term result, since the immune response may manifest itself delayed, against the background of prolonged transgene expression or when attempting subsequent correction of therapy. This is especially important for chronic and hereditary diseases, where the expected duration of the effect is measured in years. In this context, the immunological compatibility of the platform should be assessed not only by early tolerability, but also by the ability to maintain therapeutic potential in the long term.

Controlled short-term immunomodulation, which can increase the probability of successful primary transduction, is also discussed in domestic reviews [1, 5, 6]. It is emphasized that immunosuppression alone cannot be considered a universal solution. On the one hand, it does not eliminate the problem of existing neutralizing antibodies, but on the other hand, it increases the requirements for treatment safety and individual monitoring. For this reason, strategies are coming to the fore in which immunomodulation is considered not as a substitute for engineering solutions, but as their auxiliary support in carefully selected clinical scenarios.

In general, Russian reviews form the idea that overcoming immunological barriers is impossible due to one universal innovation. [1, 5, 8, 9]. There is no separate "safe" capsid, a single immunomodulation scheme, or a once-and-for-all solution to the problem of the editorial complex. The greatest potential is the combination of several levels of optimization: patient selection, molecular vector engineering, limitation of systemic exposure, control of expression duration and careful monitoring after administration. Such a multilevel model today looks like the most realistic way to increase the clinical reproducibility of gene therapy.

The current state of the Russian literature allows us to make one more observation. As gene therapy develops, the focus gradually shifts from discussing the "high efficiency of the platform on average" to finding conditions under which a particular patient will be immunologically compatible with a particular design. [4, 5, 7, 9]. In other words, the problem ceases to be solely pharmacotechnological and acquires a personalized biomedical character. That is why solutions that combine the molecular design of the vector, preliminary immunological stratification of the patient, and the choice of an administration regimen in

which the immune system does not destroy the therapeutic intent at an early stage of its implementation look the most promising.

CONCLUSION

The immunological aspects of gene therapy in the domestic literature of 2020-2025 are considered as one of the main factors limiting the transfer of even high-tech platforms into sustainable clinical practice. For viral vectors, the main barriers remain pre-existing and induced neutralizing antibodies, dose-dependent inflammation, cellular deletion of transduced cells, and difficulty in re-administration.

For genome editing systems, immunological risks are inextricably linked to genomic safety, the duration of expression of editorial proteins, and off-target DNA damage. In oncolytic virotherapy, the immune response has a dual nature: it is able to limit the persistence of the virus, but at the same time participates in the formation of a therapeutic antitumor effect.

The most promising ways to overcome barriers include preliminary immunological screening, rational choice of a vector platform, engineering modification of capsids and regulatory elements, optimization of dose and route of administration, expansion of ex vivo approaches and targeted short-term immunomodulation. In general, the development of gene therapy is moving more and more clearly towards immunologically personalized solutions, in which effectiveness is determined not only by the design of the vector, but also by the controllability of interaction with the patient's immune system.

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