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REVIEW

Memory T Cells: Investigation of Original Models with Transgenic T Cell Receptors

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Abstract—This review summarizes the research data on original mouse models developed in the laboratory of regulatory mechanisms in immunity of the Research Institute of Carcinogenesis, N. N. Blokhin National Medical Research Center of Oncology, Ministry of Health of the Russian Federation. Transfer of the genes of individual α- and β-chains of T cell receptors (TCRs) of memory cells has resulted in production of transgenic animal lines valuable for studying T lymphocyte homeostasis and patterns of formation of their activation profile markers. Investigation of the transgenic models revealed new features of immune selection and tumor progression. In particular, the fundamental property of some TCRs, termed "chain-centricity", has been confirmed; it involves dominance of one of the TCR chains during recognition of the MHC (major histocompatibility complex)/peptide complex. This property makes it possible to artificially generate a significant pool of immunocompetent T cells so it could be used in adoptive immunotherapy for oncological and infectious diseases. Transfer of the dominant active TCR α-chains provides the possibility for constructing organisms with innate specific immunological resistance to certain pathogens. The results of recent studies indicate that TCR, determining the T lymphocyte relationship with its MHC microenvironment, has an instructive role in formation of its functions and phenotype. One of these functions may be production of cyclophilin A by the cortisone-resistant memory cells localized in thymus. The evidence has been accumulated that expression of TCR with a certain structure and specificity is a sufficient condition for formation of the functional potential of memory cells in a T cell, regardless of its former interaction with antigenic MHC/peptide complexes.

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INTRODUCTION

In the middle of the last century, studies of specific immunity and immunological tolerance led immunologists to realize discrete nature of the carriers performing functions of adaptive immunity. This understanding resulted in suggestion of the clonal selection theory of immunity. According to this theory, adaptive immunity is performed by lymphocyte clones, each of which produces receptors with unique specificity. Within this theory, immunological tolerance could easily be explained by the death or inactivation of the lymphocyte clones expressing receptors for a tolerant antigen, while specific immunity – by activation and multiplication of the clones carrying receptors for an immunogenic antigen. The ability of some of these clones to persist for a long time, in turn, explained the phenomenon of immunological memory, which is the basis of vaccination methods protecting an organism against dangerous infections. Development of the T and B lymphocyte cloning techniques and further research of the structure and mechanisms

Abbreviations: MHC, major histocompatibility complex; TCRs, T cell receptors.

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of formation of the unique lymphocyte receptors have proved beyond any doubts validity of the clonal selection theory of immunity and its scientific and prognostic significance and effectiveness. Further development of the recombinant DNA technologies, as well as transfection, transduction, and transgenesis methods opened up a possibility of a wide scope of work in the field of genetic modification of lymphocytes and organisms using adaptive immunity receptor genes and investigation of acquired new properties and mechanisms of the immune system homeostasis. Full implementation of this approach in studying immunological memory was impossible due to involvement of naive T lymphocytes in the secondary immune response. Their activation by an antigen leads to acquisition of an activation marker profile similar to that of the memory cells, which makes it impossible to differentiate them and unambiguously attribute certain properties to the specific cell type [1]. This situation was further complicated by acquisition of the memory cell markers by T lymphocytes that occurred under lymphopenia in the absence of antigenic stimulation, which made the subject of research such as memory T cells even more uncertain [2]. The term "virtual memory cells" have appeared in the literature, but their real relationship to immunological memory remains unclear, along with the growing conviction that the T cells proliferating under lymphopenia are autoreactive [3, 4]. At the same time, interpretation of the results of studies focused on the memory cells obtained from the models constructed with transgenic T cell receptors (TCRs) did not provide any support to the notion that the investigated transgenic TCRs originated from the long-lived memory cells subjected to prolonged selection during the immune response *in vivo*. In this context, the T lymphocytes with a transgenic TCR that have repeatedly responded to the same TCR-specific antigen were termed "memory cells". This approach appeared to limit the ongoing research on the epigenetic component of immunological memory. Doubts have been expressed about the extent to which such models can accurately characterize real memory cells that are formed during immunological memory development. The spectrum of activation markers and functionality of the transgenic T cells reacting to the same antigen could be only indirectly associated with immunological memory [5].

The main obstacle was lack of the methods to activate memory cells selectively that would facilitate their cloning and molecular identification of TCR. One important breakthrough was discovery of the ability of memory cells to proliferate in response to allogeneic antigen-presenting cells (APC) exposed to an acute heat shock (45°C, 1 h) [6]. This feature made it possible to induce a selective memory cell immune response, which does not involve naive T lymphocytes [7, 8]. This, in turn, provided the possibility for further cellular cloning of memory T lymphocytes, obtaining T cell hybridomas from them, and identifying their T cell receptors. One of these receptors, named 1d1, which is specific to the allogeneic histocompatibility molecule class I (H-2Kb) presented on the EL-4 lymphoma cells, was subjected to gene cloning and further advanced study. Retroviral transduction of the genes of TCR chains into the thymoma 4G4 cells that do not carry their own TCR and associated CD3 complexes has shown that such transduction leads to expression of the transgenic TCR 1d1 and appearance of the CD3 complex on the cell surface [9].

TRANSFER OF THE TCR β-CHAINS GENES – CONSTRUCTION OF NEW MODELS FOR STUDYING HOMEOSTATIC PROLIFERATION OF T CELLS

The next step was to obtain animal lines with expression of individual transgenic α- and β-chains of TCR 1d1. Expression of β-chains occurs strictly according to the rules of allelic exclusion. Therefore, the β-chain transgene of the 1d1 receptor contributes to suppression of gene rearrangement and prevents expression of endogenous β-chains in the recipient T lymphocytes. The hCD2 promoter used in the work of Silaeva et al. [10] initiates transgene expression at the time of the start of gene rearrangements of endogenous β-chains. By this time, there is a small percentage of T cells, in which rearrangement of their β-chain genes has already occurred and the transgene expression is suppressed. As a result, the repertoire of T cells of the transgenic organism consists of two parts: 10-20% of T cells expressing the genes of their own rearranged β-chains, and 80-90% of T cells producing the transgenic β-chain. A more detailed analysis of the T lymphocyte characteristics in mice with the transgenic β-chain showed that the cells expressing the transgene are mainly represented by the naive T lymphocytes with the $CD44^{-}CD62L^{+}$ phenotype, whereas the T lymphocytes expressing endogenous TCR β-chains have the phenotype of activated effector cells, $CD44^{+}CD62L^{-}$ [10]. This could be easily explained by the fact that survival and homeostasis of T cells largely depend on receiving tonic signals via interaction of their T cell receptors with the endogenous molecules of major histocompatibility complex (MHC) [11]. In other words, T cells need a certain level of physiological autoreactivity for survival and homeostasis maintenance that prevents their death, just as in the thymus, where interaction of thymocytes with their own MHC/peptide complexes promotes their positive selection and prevents their "death by neglect". Lower and upper limits of this physiological autoreactivity

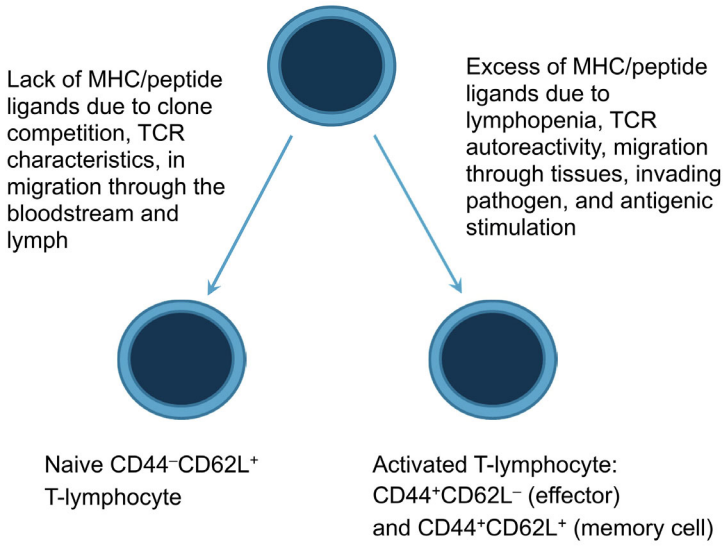

Fig. 1. A scheme of acquisition of the phenotype of naive or activated cells by a T lymphocyte, based on investigation of animals with transgenic TCR β-chain. MHC, major histocompatibility complex; TCRs, T cell receptors.

range, likely, facilitate formation of the corresponding phenotypes of naive and activated T lymphocytes. Decrease in the diversity of specificities in the TCRs with transgenic β-chains should lead to the deficit in receiving such tonic signals and increased competition of the T cells for the available endogenous MHC ligands. On the contrary, the T lymphocytes that express a variety of normally rearranged endogenous β-chains would be under conditions of relative abundance, which could be compared to the conditions of lymphopenia or immune response to an introduced pathogenic microorganism multiplying within a body. Our experiments revealed that the balance between the naive and activated T cells is maintained by their interactions with endogenous MHC/peptide complexes; it also means that the balance is mediated by autoreactivity of T cells. Moreover, we can significantly change this balance by influencing interactions of TCR with the endogenous MHC/peptide complexes – in favor of naive cells if we limit the interaction, and in favor of activated cells if we facilitate the interaction. Thus, acquisition of the naive or activated phenotype by a T lymphocyte can be considered as a result of availability of the corresponding MHC ligands. At the same time, it became clear that acquisition of the "activation phenotype" by the T lymphocyte, which is characteristic of memory cells, reveals its homeostatic interaction with the MHC molecules of the cellular environment, rather than actual "experience" of interaction with a specific antigen. A hypothetical scheme illustrating acquisition of naive and activated cell phenotypes by a T lymphocyte is shown in Fig. 1. In this case, acquisition of the phenotype of activated T cells ($CD44^+CD62L^-$ effectors or $CD44^+CD62L^+$ memory cells) could be caused by interaction with the specific antigen during an immune response, under condition of lymphopenia caused by radiation, or by significant reduction in the diversity of T cell repertoire containing different TCRs built from the endogenously rearranged α- and β-chains genes. On the contrary, acquisition of the naive $CD44^-CD62L^+$ phenotype could be associated with the lack of antigenic stimulation or limited diversity of their TCR specificity due to the invariant transgenic β-chain in their structure. It is also foreseeable that the proposed scheme may be relevant for migration of naive T cells through the lymph and bloodstream, and activated T lymphocytes – through tissues and extravascular areas of the body.

B-chains are combined with normally rearranged endogenous α-chains in both parts of the repertoire. Since the "transgenic" part of the repertoire contains an invariant β-chain, diversity of the T cell receptors in the transgenic animals is significantly reduced, which leads to moderate suppression of immune responses to alloantigens and loss of the ability to reject an allogeneic tumor. This was shown with the B10.D2(R101) (KdIdDb) mouse line, which was used as the genetic basis for expression of the 1d1 receptor β-chain transgene [10]. Normally, T lymphocytes of the wild type mice of this line respond well in the mixed lymphocyte reaction (MLR) to the stimulator cells isolated from the C57BL/10 (H-2b) or FVB (H-2q) mice. Transplantation of the EL-4 lymphoma cells originating from the C57BL/6 (H-2b) mice leads to the development of a full-fledged immune response to the lymphoma cells and their complete rejection within 12-14 days, despite the fact that antigenic differences in the transplant antigens in this case are represented only by the allogeneic molecule H-2Kb. On the other hand,

the response of T lymphocytes from the transgenic mice to the C57BL/10 (H-2b) and FVB (H-2q) stimulator cells was reduced. When the EL-4 lymphoma cells were injected into transgenic mice, the chronic immune response developed, and the immune selection of tumor cells occurred, followed by the loss of the H-2Kb molecule, tumor growth, and, as a result, death of animals 1-2 months after transplantation [12].

TRANSGENIC TCR β-CHAINS – EFFECTS ON INTERACTION OF T LYMPHOCYTES WITH TUMOR CELLS

Our research revealed that, despite the antigenic differences between the tumor (H-2Kb) and the recipient (H-2Kd) in one of the major histocompatibility antigens, the tumor development process in the animals with the transgenic TCR β-chain acquires characteristics of chronic rejection. It occurs through the stages of elimination, equilibrium, and escape of the immune response. This escape is accompanied by the loss of the specific antigen of tumor cells (the H-2Kb histocompatibility molecule) in the course of the process of resisting to the recipient immune system and selection of the weakly immunogenic variants of tumor cells under its pressure [12]. In other words, the phenomenon of rejection of an allogeneic tumor became a phenomenon of its progression under the pressure of the immune system in the allogeneic recipient due to the limited diversity of the repertoire of T cell receptors. Our data show that effectiveness of the antitumor immune response could depend on the range and diversity of the repertoire of T cell receptors. Theoretically, this diversity is very high and amounts to ~10^{18}. Only a small part of it is realized, since the total number of T cells in the human body is ~4-5 × 10^{11} [13, 14]. This means that the process of overcoming of immunological defense by the tumor and limited successes and achievements in the field of tumor immunotherapy could be due to the deficiency in the specificity of T cell receptors necessary for the successful immunological recognition of tumor neoantigens. It cannot be ruled out that the development of approaches to artificially expand the repertoire of receptors of cells of the adaptive immune system could help to overcome this obstacle. This, in turn, indicates that success in the development of tumor immunotherapy methods should be expected in the area of delivering of the genes of T cell receptors with required specificity to the patients, i.e., a type of passive immunotherapy. Fundamental aspects of the revealed patterns are equally important, as they shed light on the nature of alloreactivity, a phenomenon that appeared to be critically dependent on the diversity of the T lymphocyte receptor repertoire in the recipient. The obtained results support the hypothesis that the T lymphocyte receptors have an intrinsic ability to react with any of the allelic variants of MHC molecules [15]. According to it, the allogeneic immune response is a combination of the responses of individual recipient T lymphocyte clones to foreign MHC/peptide complexes that did not participate in the negative selection of T lymphocytes in the thymus and formation of their repertoire. On the other hand, negative selection of T cells in the thymus as a result of "strong" interaction with their own MHC/peptide complexes could be considered as the first and last allogeneic reaction of the emerging repertoire of T lymphocytes to the self MHC molecules [16].

TRANSFER OF THE GENES OF TCR α-CHAINS AND CHAIN-CENTRIC T CELL RECEPTORS – PROSPECTS FOR DEVELOPING NEW TECHNOLOGIES OF IMMUNOLOGICAL PROTECTION

Transgenic T cell receptors have become an invaluable tool for fundamental research on the mechanisms of immune system development and adaptive immunity functioning. Availability of such research tools for immunologists has provided opportunities for visualizing the processes of intra-thymus selection of T lymphocytes, formation of the ability of immune system to distinguish between the "self" and "non-self", and of the processes of development and differentiation of T lymphocytes, and the mechanisms of interaction of T cell receptors with MHC/peptide complexes [17].

A number of significant features of TCR biology prevented the use of this tool in practice. These receptors are known to be heterodimers consisting of α- and β- or δ- and γ-chains. The fate of the developing T lymphocyte is strictly determined by the particular V, D, and J segments forming a functional gene, the sequence of which will be in the reading frame. During the intra-thymus development, rearrangement of the gene segments of the β-, δ-, and γ-chains begins first. Formation of the functional variant of the β-chain gene blocks irreversibly further rearrangements of other genes: the functional gene remains active, while expression of recombinases and rearrangements of other β-, δ-, and γ-chains stops, and they enter the region of condensed chromatin. At this stage, a T lymphocyte could become either an αβ- or γδ-T cell; any such selection has a strictly alternative character [18]. Attempts to interfere with this process and introduce a transgenic β-chain into the T lymphocyte negatively affect the immune system – rearrangements of the genes of endogenous β-chains stop. The mechanism of allelic exclusion is activated,

which is accompanied by the dramatically reduced diversity of T cell receptors. As a result, most of the receptors contain an externally introduced invariant transgenic β-chain, which leads to suppression of adaptive immunity [12, 19, 20]. Appearance of the functional β-chain gene leads to expression of a gene, protein product of which combines with the surrogate α-chain, forming a pre-TCR localized in the endoplasmic reticulum that possesses an autocatalytic ability to generate the signal for further T lymphocyte development. This signal initiates the second wave of recombinase expression and rearrangement of the α-chain genes, which can occur repeatedly by connecting distally located V and J segments. The secondary re-arrangement occurs if the previous combination did not form a functional gene or the formed α-chain is non-functional at the protein level, i.e., unable to ensure the TCR interaction with MHC molecules of the thymus medullary epithelium; as a result, the thymocyte cannot pass the selection. Unlike β-chains, allelic exclusion of α-chain genes is not strict, and their rearrangement could lead to formation of functional genes on both chromosomes and, accordingly, to a T lymphocyte with two αβ-T cell receptors [21]. At this time point, the fate of the T lymphocyte depends critically on whether the αβ heterodimer can interact with the MHC class II molecules located on the surface of the thymus epithelium. Stable interaction of TCR with the MHC class II molecules mediated selection of the CD4 coreceptor by the developing T cell. Weak and unstable interaction of TCR with the molecules of this type triggers suppression of CD4 expression and commits to the development towards T cells with the CD8 coreceptor. Complete absence of interaction with the MHC molecules results in the T lymphocyte death, which is called metaphorically “death by neglect”.

Nearly a decade ago, it became clear that the significant part of the repertoire of T cell receptors has the property of chain-centricity, i.e., one of the TCR chains is dominant in the recognition of MHC/peptide complexes [22, 23]. Incorporation of the gene of the single dominant-active chain of the chain-centric TCR into the cell creates a unique possibility to influence its interaction with the MHC/peptide complexes, thus giving the T cells a new specificity. It is worth noting that modification of T cells with the TCR α-chains has many significant advantages. Due to the absence of allelic exclusion of the α-chain genes and the possibility of expressing two TCRα in one lymphocyte, transfer of the α-chain gene could dramatically expand the diversity of the repertoire of T cell receptors without causing systemic suppression of an immune response. This possibility was realized by constructing the transgenic mouse line, in which α-chain of the chain-centric TCR (1D1a) was incorporated in the wild-type mouse genome as a part of the pTα cassette vector, which provides tissue- and stage-specific expression of the transgene. The transgenic α-chain originated from the TCR T cell hybridoma of memory cells specific to the H-2Kb alloantigen of the EL-4 lymphoma cells. Compared with the wild-type mice, the transgenic animals had increased CD3 expression on the surface of double negative thymocytes of the DN2 and DN3 stages, undergoing the processes of gene rearrangement and selection of functional β-chains. Moreover, increase in the proportion of $CD8^+$ cells with the phenotype of central memory cells ($CD44^+CD62L^+$) and effectors ($CD44^+CD62L^-$) was observed in the peripheral lymphoid organs. Similarly to the effect of the transgenic β-chain expression on the surface phenotype of T cells described above, the data obtained in the analysis of the 1D1a mice indicate an instructive role of the TCR structure in the formation of the T lymphocyte phenotypic properties.

The most unexpected finding was that, although the transgenic mice expressed the α-chain of the original TCR without its β-chain, they had developed immunity to the EL-4 lymphoma cells. The animals rejected the transplanted tumor cells within 2-3 days, which is typical for the immunized animals with memory cells for the immunizing antigen (the H-2Kb histocompatibility molecule). In MLR, the peripheral lymphocytes of transgenic animals produced enhanced proliferative responses to the stimulator cells carrying H-2Kb histocompatibility molecules on their surface. Thus, the TCR structure, altered by the transgenesis of only one α-chain, determined the functional properties of T cells in the transgenic organism. At the same time, immune responses to third-party antigens in the 1D1a mice were comparable to the wild-type animals. Thus, expression of the transgenic TCR α-chain does not suppress immune responses of the 1D1a mice to other antigens. During the study, we demonstrated that the transgenic α-chain originating from the chain-centric TCR can pair with endogenous β-chains and enter the surface of T lymphocytes in the form of heterodimers. As a result, the repertoire of T lymphocytes in the transgenic animals is represented fully or partially by the lymphocytes carrying an additional T cell receptor on the surface, the specificity of which is determined by the transgenic α-chain [23].

These studies of the chain-centric TCR with dominant active α-chains [23-26] provide a theoretical basis for creating organisms with innate specific immunological resistance to certain pathogens.

CHAIN-CENTRIC T CELL RECEPTORS AND ADOPTIVE IMMUNOTHERAPY

Studies of the 1D1a transgenic mice have shown that these animals have enhanced innate antitumor

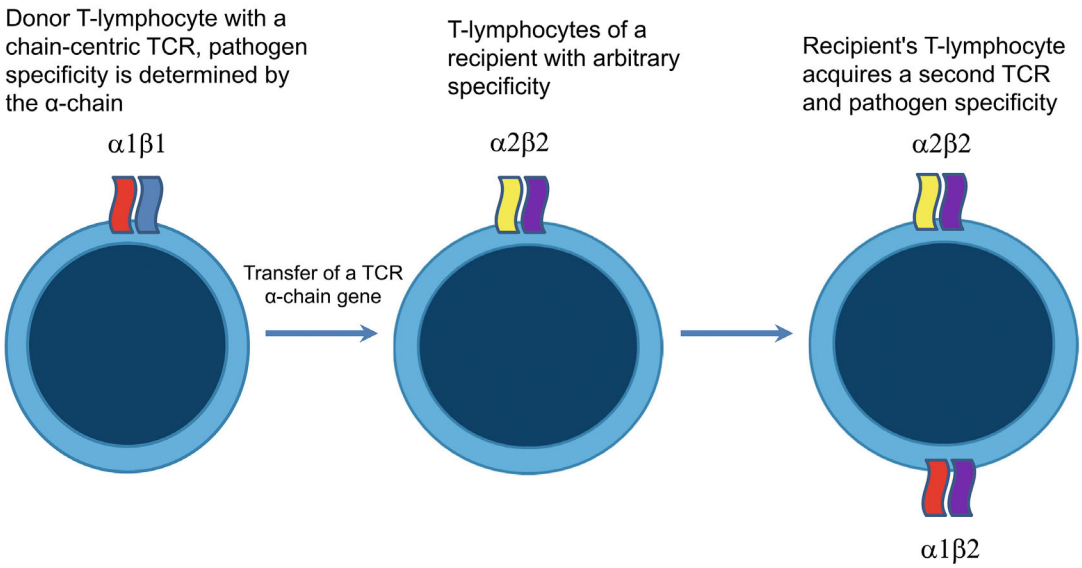

Fig. 2. Gene transfer of the dominant active α-chain of the chain-centric TCR into recipient's T lymphocytes or into the zygote results in the repertoire of T lymphocytes with the additional TCR specificity. TCRs, T cell receptors.

immunity due to the expression of the dominant-active α-chain of the chain-centric tumor-specific TCR.

Considering that transfer of the dominant α-chain 1D1a gene to the cells redirected the repertoire of T cells to fight tumor cells, a similar effect could be expected with *in vitro* modification of normal T lymphocytes with the same dominant α-chain. Indeed, using retroviral transduction, we generated T cells that expressed 1D1a and showed protective and therapeutic effects against the EL-4 lymphoma cells after adoptive transfer to the B10.D2(R101) mice [23]. Figure 2 shows how transduction or transfer of the α-chain gene of the chain-centric TCR creates an additional receptor and thereby modifies the specificity of the recipient T lymphocytes.

These results were confirmed with the infectious model. The mouse memory T cells for *Salmonella typhimurium* antigens were generated in the *in vivo* system, with subsequent re-stimulation with antigens of the same bacterium *in vitro*. To identify the sequences of TCR clonotypes that responded to re-stimulation, a new-generation sequencing (NGS) method was used to obtain repertoires of α-chains of the TCR in the memory T cells stimulated by the model pathogen and in the memory T cells without stimulation. The α-chain variants, frequency of which increased in the TCR repertoire after re-stimulation with the Salmonella antigens, were individually transduced into the normal T lymphocytes. The dominant- active TCR α-chains were identified by the ability of modified T cells to respond by proliferation to the Salmonella antigens *in vitro*. About 20% of the TCR repertoire of memory cells that responded to Salmonella antigens exhibited chain-centricity and contained the dominant-active α-chain [24, 25]. Similar results were obtained in the research investigating response to the *Listeria monocytogenes* antigens [27].

Thus, chain-centric TCRs were found to comprise a significant proportion of the T cell receptors that respond to bacterial pathogen antigens. Moreover, we developed an algorithm for the chain-centric receptor searching, according to which cloning of the antigen-specific T cells is not required. Since such cloning is usually laborious and time-consuming, the above-mentioned approach is quite desirable to be applied in practice.

The dominant-active α-chains of the chain-centric T cell receptors are promising candidates for the use in adoptive immunotherapy of oncological and infectious diseases, similar to the CAR-T chimeric antigenic receptors used in oncology. Advantages of the chain-centric TCR include the lack of immunogenicity characteristic of the chimeric proteins and the absence of the negative trans-completion effects on the signal transmission into the T lymphocyte, which are unavoidable in the case of transduction of both TCR chains. Moreover, it involves a simple and clear search algorithm along with the possibility of direct targeting of the immune response to the unique tumor neoantigens [28, 29]. To assess feasibility of introduction of this approach into clinical practice, a preclinical study was conducted assessing biosafety of the T cell products modified with the chain-centric TCRs according to the following parameters: physiological condition of animals, acute toxicity, immunotoxicity, allergenicity, mutagenicity, tumorigenicity, and pharmacokinetics. The conducted studies indicate the safety of the transgenic constructs injected with T cells.

At the same time, introduction of T lymphocytes activated during transduction in order to increase effectiveness of the procedure could lead to nonspecific allergic reactions in the recipient [30]. Studies evaluating the safety of the transfer of chain-centric receptors, characteristics of transgenic animals, and their differences from the wild-type mice were also conducted, and the functional condition of the immune system of the 1D1a transgenic mice expressing the dominant-active α-chain of the chain-centric TCR specific to the H2-Kb molecule was analyzed. Neither autoimmune diseases due to the random pairing of transgenic TCRα with endogenous TCRβ variants nor considerable disturbance of systemic homeostasis were observed in the age dynamics of these mice. It is worth noting that the TCRα transgene expression could delay thymus involution and maintain the TCRβ repertoire diversity in the old transgenic mice. Despite the notable enhancement of the specific immune response in the 1D1a mice, responses to the third-party alloantigens were not affected, indicating that expression of the transgenic TCRα did not limit the immunoreactivity of the transgenic mice [26].

CHAIN-CENTRIC T CELL RECEPTORS AND IMMUNOLOGICAL MEMORY

Along with the data accumulation in the research field, there is a growing recognition of the fact that chain-centricity is one of the basic properties of the significant part of T lymphocyte receptors. The question arises: why this feature has not been investigated explicitly in the scientific literature over the last half-century? Our analysis of the available sources has shown that there are numerous indications of this characteristic in the literature, but indirect in most of the cases, fragmented, and not properly interpreted [29]. The reason could be in the artificiality of the discovered phenomenon since the transfer of the genes of TCR chains does not occur in nature, and functional value of the chain-centric receptors in the naturally formed repertoire of T cells is not clear. Nevertheless, simultaneous expression of two TCR α-chains due to successful rearrangement of the genes encoding them on both chromosomes and incomplete allele exclusion has been known, although, according to the previous estimates, it is a rare event (1-10%) [31]. Recent estimates of the frequencies of T cell expressing two T cell receptors simultaneously are dramatically higher and is ~16% [32, 33]. Proportion of these cells increases considerably with age [34], as well as in some autoimmune diseases [35]. In the acute phase of the response to infection with lymphocytic choriomeningitis virus (LCMV), up to 60% of the virus-specific T cells express double TCR. Moreover, after the infection is complete, memory cells contain increased frequencies of the $CD4^+$ T cells with two TCRs [36]. It is also interesting that in the patients with Kawasaki syndrome who have undergone immunoglobulin therapy, the proportion of the $CD8^+$ memory cells with two TCRs increases [37].

Thus, simultaneous expression of two T cell receptors has a functional significance and is involved in formation of immunological memory, but its relationship with the effect of chain-centricity of TCR α-chains is not obvious. Considering that we detected the first chain-centric TCR in the long-lived memory cells, it is likely that the frequencies of such TCRs are rather low at the earlier stages of the immune response. Presumably, this could be a reason why chain-centric TCRs were not detected in the 1990s, at the peak of investigations focused on the structure and functions of TCR. At the moment, it is not entirely clear whether chain-centricity is an intrinsic feature of some T cell receptors or whether it is related to selection of the repertoire of memory T cells. Our work aimed at identifying frequencies of the chain-centric TCR during formation of immunological memory would shed light on this fundamental issue. The results of the T cell repertoire study in the animals immunized with allogeneic tumor cells confirm indirectly the hypothesis of the chain-centric TCR expression mainly by the memory cells. Using an experimental model of induction of the antitumor immune response, sequential changes in characteristics of the TCR repertoire of T cells involved in the primary and secondary immune responses to allogeneic tumor antigens were studied. Bioinformatics analysis of the TCR repertoire showed that, compared with the primarily activated effectors, re-stimulation of memory cells with the specific antigen led to their enrichment with the clonotypes that expressed TCR α-chains with high potential cross-reactivity and the increased strength of interaction with both MHC molecules and docked peptides. No significant changes in the psycho-chemical characteristics of TCRβ were observed in the reactivated clonotypes of memory T cells, which may indicate the dominant role of TCRα in the secondary allogeneic immune response. This effect could be due to the increase in the frequencies of chain-centric TCRs during the memory cells formation [38].

IMMUNOLOGICAL MEMORY AND CYCLOPHILIN A

In the recent decades, extracellular cyclophilin A and its biological activity have been objects of serious studies in our laboratory. Interest in this protein first arose when T. V. Anfalova attempted to cultivate *in vitro* thymic T lymphocytes (thymocytes) of mice

treated with high doses of hydrocortisone, causing apoptosis of more than 98% of thymocytes. A minor part of the cells remained viable, proliferated in the *in vitro* culture, and secreted into the medium an unknown factor with the properties of radioprotector, significantly prolonging life of the animals that received sublethal doses of radiation. This effect was found to be associated with the increased migration of stem cells from the bone marrow of irradiated animals to their peripheral lymphoid organs. It was reproduced *in vitro*: the factor of cortisone-resistant thymocytes induced chemotaxis and migration of the hematopoietic cells of different linages, including T cells, B lymphocytes, monocytes, granulocytes, and dendritic cells. On this basis, a test suitable for isolating and purifying the target protein was developed. Further purification and identification of the factor of cortisone-resistant thymocytes via Edman degradation allowed identifying cyclophilin A [39]. Recombinant production of the human cyclophilin A with demonstration of its biological effects, partially reproducing the effects of the factor of cortisone-resistant thymocytes, confirmed our previous findings [40, 41]. Further investigation of the cyclophilin A biological activity attracted a lot of attention when its antitumor and antimetastatic effects were revealed [42]. We have shown pro-inflammatory effects of cyclophilin A in the study of expression of the genes of acute phase inflammatory proteins in the animal liver after administration of high doses of this protein [43]. Moreover, cyclophilin A, similar to the well-known proinflammatory factor TNFα, appears to exhibit an embryotoxic effect, disrupting embryonic development during organogenesis [44]. Thus, cyclophilin A is involved in regulation of acute inflammation, but its role and part in the regulation of this response has yet to be established.

A wide range of studies have revealed the involvement of the extracellular cyclophilin A in inflammatory and autoimmune diseases. The available data are presented and discussed in the reviews by Kalinina et al. [45] and Xue et al. [46]. The function of this protein in immunoregulation involves polarization of the immune response towards the type 1 T helper cells [47].

Two areas (pieces) of our studies, the investigation of memory cells and of cyclophilin A, which appear to be not closely related, suddenly fit the puzzle when we determined that the cortisone–resistant thymocytes (producers of cyclophilin A [39]) contain memory T cells, comprising the major part of this cell population. Administration of hydrocortisone at a dose of 2.5 mg per animal to the pre-immunized animals led to the 95-98% decrease in the thymus cellularity, but at the same time to the multifold increase in the proliferative response of the remaining lymphocytes in the thymus to the immunizing antigen. This indicated enrichment of the thymocyte population with the mature cortisone-resistant lymphocytes with the antigenic specificity [48]. Most likely, exposure to high doses of hydrocortisone enriches the thymus with T lymphocytes that express high levels of antiapoptotic molecules (which was repeatedly noted in memory cells) [49, 50].

Cyclophilin A is known to play an important role in the negative regulation of apoptosis [51, 52]. Moreover, there are common features in the signaling pathways involved in the memory cell differentiation and regulation of oxidative stress by cyclophilin A [53, 54]. It was also shown in the recent study that in the course of the signal transduction through TCR, cyclophilin A regulates activity of the ZAP70 tyrosine kinase, which is important for activation and differentiation of T cells [55]. According to the data available to date, the increased level of cyclophilin A appears to be part of the epigenetic profile of the memory T cells.

The reason and biological significance of the memory T cells emergence in the thymus remain enigmatic. The thymus is traditionally considered to be a central lymphoid organ focused on the export of T lymphocytes formed in it. The major function of the thymus involves creation of a microenvironment that ensures expression of RAG-1 and -2 recombinases responsible for recombination of the TCR gene segments in T lymphocytes. Therefore, memory T cells are assumed to migrate to thymus to edit α-chains of their TCR, which would have the following consequences: (i) maintenance of the diversity of the T lymphocyte receptor repertoire on the periphery due to the changes in TCR of memory cells that formed in excess during the immune response and became “unnecessary” upon its completion; (ii) formation of TCR α-chains that ensure high affinity of the memory T cell receptors to the antigen. In other words, TCR editing of a memory cell could lead to the formation of either a receptor with a new specificity or a chain-centric TCR with an increased interaction affinity of its α-chain to the original (immunizing) MHC/peptide complexes [38]. Based on the data available to date, we believe that the TCR editing, leading to the formation of a chain-centric receptor, could be one of the mechanisms of T cell functional avidity maturation, similar to the affinity maturation of the B-lymphocyte receptor. Figure 3 presents the hypothetical scheme (based on the data results described in this work) of the T lymphocyte repertoire formation and further transformations of T lymphocytes during the immune response. This scheme differs from the other models of linear differentiation of memory cells from naive precursors into effectors and further into memory cells, since it assumes formation of memory

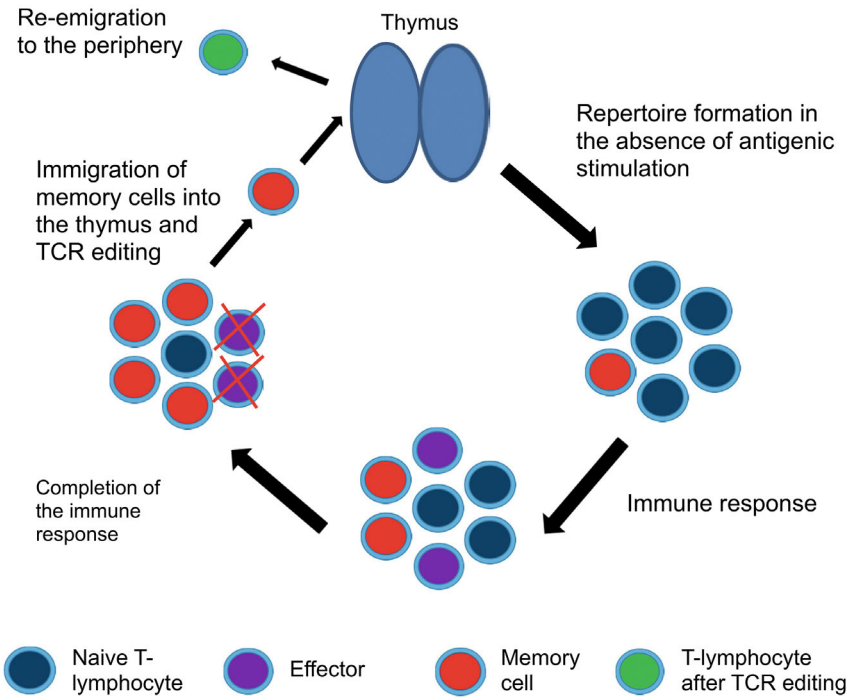

Fig. 3. Memory cells during the antigen-independent formation of the T cell repertoire in the immune response and its completion. TCRs, T cell receptors.

cells regardless of the initial contact with the antigen. This formation is determined by the TCR structure and features of its interaction with the microenvironment of the T lymphocyte. In this case, there are T cells, which perform in the secondary immune response functions that we call immunological memory. Our experiments showed that T cells with the properties of memory cells pre-exist before the contact with the antigen. They appear to be "not visible" in the primary immune response due to their small number and long time required for their antigen-specific clones to grow to the experimentally detectable size. They could also be detected in the primary response if the α-chain transgene of the chain-centric TCR is introduced into an organism. The presented scheme of the memory cell formation, regardless of antigenic stimulus, is in agreement with the existing ideas about the constitutive formation of memory cells and early determination of their fate [56, 57], which now has direct experimental confirmation [23, 26].

Based on the results of our studies of the cortisone-resistant thymocytes, we suggest that memory cells could be producers of cyclophilin A, which promotes such "secondary selection" of T lymphocytes in the thymus.

Exhaustive evidence of this is not yet available, but its exploration may be an important area of further research. It has not been ruled out that cyclophilin A could serve as a diagnostic marker for the detection and identification of memory cells.

CONCLUSION

In general, it could be concluded based on the results of our investigations that the relevant study of immunological memory should be based on techniques that provide an assessment of their specificity, functional properties, and protective effects. It has been generally recognized that expression of the genes of activation markers in T lymphocytes mostly reflects general homeostatic interactions of the lymphoid system with the microenvironment rather than an immune response to foreign antigens, and cannot be directly attributed to the properties characterizing cells of immunological memory. Memory cell cloning with further identification of their TCRs allowed us to reveal a relationship between the formation of immunological memory and accumulation of T lymphocytes with chain-centric receptors. The notion about such property of the part of TCRs has not been so far fully clarified. Advances made during the study of such receptors are promising for the development of new methods of adoptive immunotherapy of cancer and infectious diseases, as well as for creating organisms with innate specific resistance to bacterial and viral pathogens. The obtained data of the pilot study indicate possible association between immunological memory and cyclophilin A production. In the future, it seems very important to clarify the role of cyclophilin A in the formation and functioning of memory T cells and to obtain data confirming or rejecting

the hypothesis implying the existence of the mechanism of "secondary selection" in the memory cells in the thymus.

Contributions. D.B.K., A.A.K., and L.M.Kh. participated in the general discussion of the concept, ideology, and work plan scheduling; D.B.K. prepared the manuscript; A.A.K. and L.M.Kh. edited the manuscript and performed its technical design.

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