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REVIEW

Revision of the Antigen Receptor of T-Lymphocytes

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Abstract—This review considers a crucially new mechanism of T-cell antigen-recognizing repertoire formation. It includes the revision of T-cell antigen receptor (TCR), which implies the secondary rearrangement of TCR genes in peripheral T-lymphocytes and surface expression of a new antigen receptor with altered specificity. Factors and mechanisms involved in the induction of this process have been analyzed. Certain attention is paid to a possible role of TCR revision in the formation of peripheral tolerance in the processes of "avidity maturation" of T-lymphocytes during immune response and also negative consequences related to appearance of potentially autoreactive clones in the periphery.

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During immune response, T-cells recognize foreign antigens due to expression of antigen-specific receptors (T-cell receptor, TCR). The antigen receptor of mature T-lymphocyte consists of two covalently bound chains, αβ or γδ. Genes encoding all four TCR chains have a mosaic structure and their functional expression is preceded by rearrangement, forming TCR of various specificity; this determines the antigen-recognizing repertoire of T-lymphocytes [1, 2].

In the dominating population of T-cells, αβT-lymphocytes, TCR formation occurs during thymic development. According to generally accepted hypothesis, the αβT-lymphocyte leaving a thymocyte carries the final TCR variant, which is not subjected to further correction, and all its daughter cells appearing on the periphery express antigen receptor of the same specificity [1]. Facts inconsistent with this hypothesis have been known for a long time, but until recently they have been considered as exceptions originating as the result of alteration of maturation and functioning of T-cells. However, there is increasing experimental evidence indicating the possibility of repeated induction of rearrangement processes in peripheral T-lymphocytes accompanied by formation of TCR with new specificity [3-5]. Since these processes appear in various T-cell subpopulations ($CD4^+$ and $CD8^+$) and they are not necessarily accompanied by pathological conditions, we may suggest that they do not represent a rare event. It is possible that these rearrangements are rules rather than exceptions. Data on the revision of TCR on periphery give a new consideration for mechanisms responsible for formation of the antigen-recognizing repertoire of T-lymphocytes. These data also give a new interpretation of processes that have been thought to be unassociated with the rearrangement of TCR genes, for example: induction of T-cell anergy (loss of T-cell activation in response to antigen) or so-called "avidity maturation" of T-lymphocyte during immune response. This review analyzes such processes.

CLASSIC MECHANISM OF TCR GENE REARRANGEMENT

Genes encoding variable domains of TCR chains as well as genes encoding immunoglobulins are formed during somatic recombination due to association of discrete DNA segments of three various classes: V (variable), D (diversity), and J (joint). So this recombination is defined as V(D)J recombination [6]. Certain DNA loci may contain up to a hundred variants of coding segments of a given class; random association of these segments during recombination is one of the basic mechanisms responsible

Abbreviations: CD) clusters of differentiation; MBP) myelin basic protein; Mtv) mammary tumor virus; NOD) non-obese diabetic (mice); RAG) recombination activating genes; RSS) recombination signal sequence; TCR) T-cell receptor; TdT) terminal deoxyribonucleotide transferase.

for diversity of TCR molecules and therefore the antigen-recognizing repertoire of T-lymphocytes. The recombination process includes two steps: DNA cleavage and repair of the breaks formed. The coding gene segments (V, J for α-chain and V, D, J for β-chain) are flanked by recombination signal sequences (RSS). These sequences consist of two conservative sites: a heptamer (5′-CACAGTG-3′) and nonamer (5′-ACAAAAACC-3′), separated by a spacer of 12 or 23 bp (Fig. 1). Effective recombination is possible only between the genetic segments flanked by RSS with various length of the spacer (12-RSS and 23-RSS), known as 12/23 rule [6, 7]. All coding segments of a given class (V, D, or J) carry identical RSS, and due to such organization V-segment may be joint only with D-segment of the same locus but not with the second V-segment (Fig. 1a). Heptamers and nonamers of 12-RSS are complementary to the nucleotide sequences of 23-RSS, and during recombination they are associated with corresponding sequences and form the synaptic complex (Fig. 1b). Recombinases RAG-1 and RAG-2, protein products of recombination activation genes (RAG), are obligate elements of the synaptic complex [8, 9]. These proteins exhibit endonuclease activity. They specifically bind to RSS, initiate double strand breaks of DNA between coding gene segment and corresponding RSS [10], and excise DNA fragment between two individual segments.

The second step of recombination is break repair. DNA cleavage results in formation of two types of terminal structures: opened 5′-phosphorylated RSS end (signal ends) and ends of coding DNA segments, in which terminal nucleotides of two DNA strands are covalently ligated with hairpin formation (the coding ends). Ligation of signal and coding DNA ends involves universal mechanisms of DNA repair similar to those employed by a cell in response to the action of various damaging agents such as ionizing and ultraviolet radiation, reactive oxygen species, etc. [11]. In the repair process, signal ends are accurately ligated without loss of nucleotides, whereas hairpins undergo processing before ligation: endonuclease nicks one of the DNA strands either between terminal nucleotides or (more frequently) near them. In the latter case hairpin opening results in formation of palindrome nucleotide inserts. Terminal deoxyribonucleotide transferase (TdT) is responsible for non-template nucleotide addition to the nicks; this makes some contribution (together with formation of DNA palindrome sequences) to formation of the huge diversity of immunoglobulin and TCR specificities [7, 12].

Let us consider the role of RAG-1 and RAG-2 in gene rearrangement in more detail. First, in contrast to other factors involved in the recombination process, these proteins are specific for lymphocytes and they determine unique somatic recombination in these cells. Second, expression of RAG proteins is the ultimate precondition required for rearrangement of TCR genes. Blockage of corresponding genes in mice results in the absence of V(D)J-recombination and total arrest of T-cell development at the stage preceding TCR β-chain rearrangement and finally to development of complex combined immunodeficiency [13, 14]. Third, after completion of TCR gene rearrangement and formation of functional TCR, expression of both recombinases are irreversibly terminated [15], and detection of RAG-1 or both RAG-proteins is widely used as a criterion of existence of the rearrangement process.

At the first stage of recombination (DNA cleavage) RAG proteins recognize RSS, and due to their endonuclease activity they form double strand breaks between coding segments of the gene (V, D, or J) and the corresponding RSS [16, 17].

It should be noted that only RAG-1 exhibits endonuclease activity. It specifically binds to a nonamer of RSS [18], whereas RAG-2 cannot directly interact with DNA [19], and it binds to the RAG-1·DNA complex [20]. The putative role of RAG-2 in the signal complex is still discussed. According to most data, RAG-2 is required for successful rearrangement. For example, it significantly increases affinity and specificity of binding of RAG-1 to DNA [19, 21]. RAG-2 is also essential for manifestation of endonuclease activity of RAG-1 [18]. However, some data suggest that RAG-1 may exhibit high affinity and specific binding to RSS and RAG-2 plays a minor role in this process [18].

The RAG-1·RAG-2 complex contains many components, and there are contradictory data on the stoichiometry of RAG proteins in it. In this complex RAG-1 usually exists as a dimer [22, 23], and these two proteins play different roles: one contacts with nonamer, whereas the other one occupies the heptamer at the cleavage site [21, 22]. However, later study revealed that presence of at least three RAG-1 proteins [24]. RAG-2 was found in the signal complex as monomer [22] or dimer [23, 24]. These controversial results on stoichiometry reflect not only different methodologies. Functioning of the signal complex and recombinase activity are significantly influenced by factors of intracellular microenvironment, which are not only cell specific but also they depend on functional states of these cells. For example, promoter of the mouse RAG-2 gene is differently regulated in T- and B-lymphocytes [25], and effectiveness of RAG functioning directly depends on type and bivalent ion concentrations in the recombination site [16, 26-28]. It is possible that *in vivo* composition of RAG-1·RAG-2 complex depends on cell types, their differentiation stage, the presence (and levels) of activation, and some other preconditions. This should be taken into consideration during analysis of the above-mentioned contradictory data on the role and importance of RAG-2 for the rearrangement process. It is also possible that in some cases rearrangement does not require the second recombinase.

After DNA cleavage RAG proteins remain within the signal complex where they exhibit at least two differ-

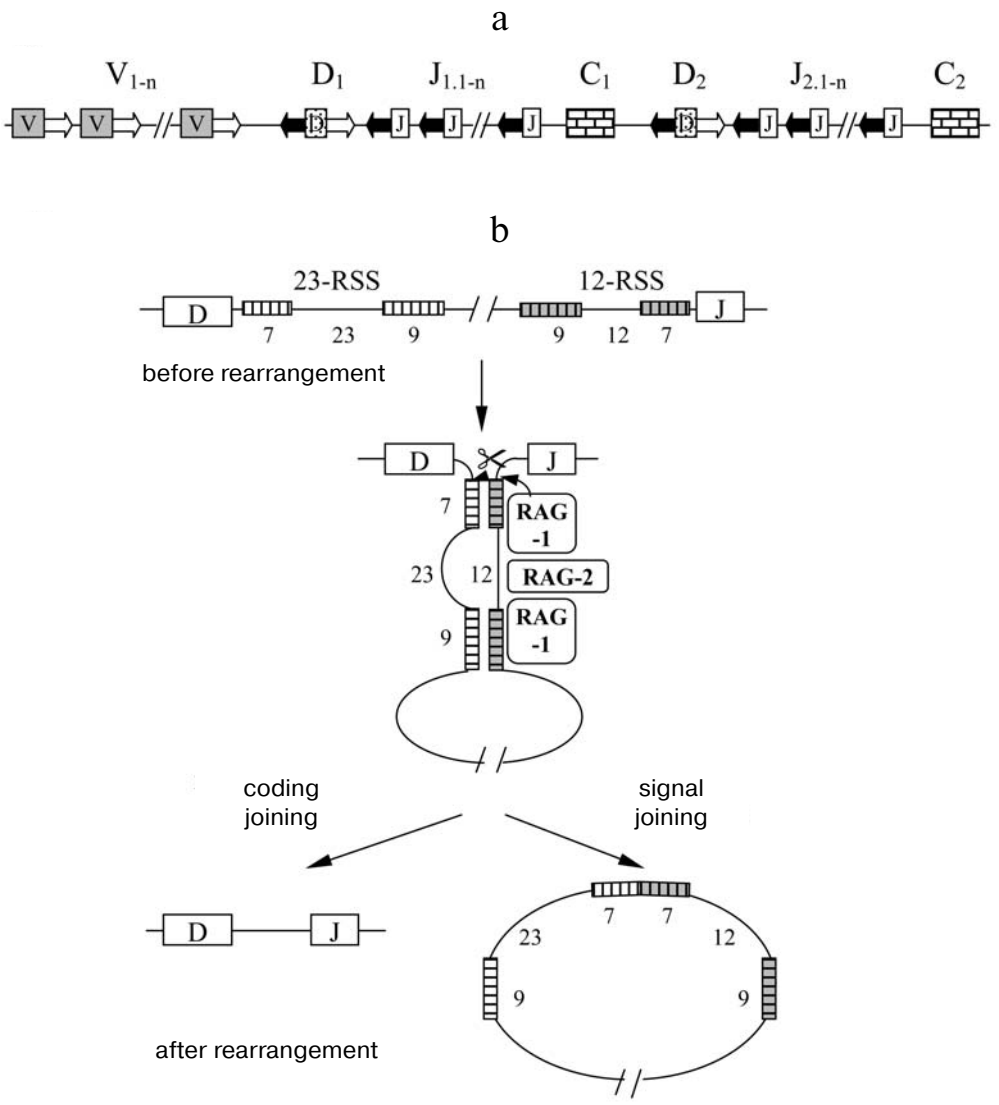

Fig. 1. Mechanisms of rearrangement of the gene encoding β-chain of T-lymphocyte antigen receptor. a) Schematic presentation of TCRβ-locus. Arrows indicate RSS sequences flanking coding segments of this gene: opened and closed arrows designate 23-RSS and 12-RSS, respectively. b) Hypothetic scheme of rearrangement using D- and J-segment joining as an example.

ent functions during the next phase of V(D)J-recombination: break repair. The first function consists in structural integrity of the post-cleavage complexes. These proteins maintain integrity of RAG·DNA complex for prevention of degradation of coding, and signal DNA ends up to the moment of their joining [23, 29]. The second function is the catalytic one. RAG proteins, due to their endonuclease activity, are involved in processing of the coding ends, by nicking DNA and opening hairpins [30]. Removal of these proteins right after DNA cleavage in a cell-free system prevents coding joint formation; this indirectly underlines an important role of recombinases at the stage of DNA repair [31].

Normally expression of RAG proteins occurs in immature T- and B-lymphocytes. In αβT-cells, it is registered at the stage of thymic development in which two waves of recombinase expression have been recognized [32]. Initially RAG-1 and RAG-2 appear at the stage of $CD4^-8^-$ cells; this is accompanied by V(D)J-rearrangement of β-chain TCR genes. Appearance of functional β-chain on membrane, association of this chain with surrogate α-chain, and activation of pre-TCR initiate cell proliferation and temporal RAG suppression. This has a logical explanation: immature T- and B-cells express both proteins only in G1 phase of the cell cycle. At S, G2, and M phases, the level of RAG-2 is 20-times lower due to RAG-2 phosphorylation by cyclin-dependent kinases followed by its subsequent degradation [33]. The second wave of RAG protein expression is registered at the subsequent stage of thymic development in resting $CD4^+8^+$

cells. This wave is related to rearrangement of α-chain, and it terminates by expression of functionally competent membrane αβTCR. At subsequent stages of T-cell selection, interaction of such receptor with antigen induces subsequent differentiation of cells into $CD4^+8^-/CD4^-8^+$ lymphocytes, and this irreversibly suppresses expression of both recombinases [15]. This phenomenon has been reproduced in *in vitro* systems on TCR cross-linking or protein kinase C activation by phorbol esters [15, 32].

Thus, it is believed that in the absence of some impairments of thymic development, mature T-lymphocytes leaving the thymus do not express RAG and do not rearrange TCR [34, 35]. Consequently, specificity of antigen receptor formed in the thymus remains unchanged and all T-lymphocyte descendants appearing on the periphery during immune response express the same TCR.

Nevertheless, recently a series of interesting reports appearing in the literature clearly indicate that there are many exceptions from this rule, and effective rearrangement of TCR genes may take place not only in thymus but also in the periphery.

REARRANGEMENT OF TCR GENES IN PERIPHERAL T-LYMPHOCYTES

The first report on this subject was published in 1998 [3]. The authors, McMahan and Fink, analyzed induction of tolerance of mature T-lymphocytes to endogenous superantigen encoded by mouse mammary tumor virus 8 (Mtv-8). This superantigen can exhibit weak interaction with T-lymphocytes carrying a sequence of TCR β-chain variable domain encoded by a certain gene segment, Vβ5. So $V\beta5^+$TCR transgenic mice were used in that study. These authors found two different pathways of induction of tolerance to Mtv-8 in the $CD4^+$-subpopulation of peripheral T-cells. Most $V\beta5^+CD4^+$ T-lymphocytes were eliminated, causing marked inversion of the $CD4^+/CD8^+$ T-cell ratio in such animals. However, some proportion of lymphocytes underwent an alternative process leading to appearance of $CD4^+$ T-cells tolerant to the viral antigen. This alternative pathway was characterized by sequential loss of expression of transgenic membrane $V\beta5^+$TCR, induction of RAG-1, RAG-2, and TdT, and appearance of endogenous TCR β-chain. This pathway was defined as TCR revision. Subsequently, this study was significantly extended and concretized. Based on comparison of expression profiles of different Vβ variants, it was demonstrated that the newly formed TCR-repertoire exhibits nearly the same diversity as in non-transgenic mice [36]. Later studies revealed that TCR revision depends on CD28 expression, the presence of B-lymphocytes [37], and it is not limited by recent thymic emigrants, because the revision can be induced in cells that left the thymus two months earlier [38]. Moreover, TCR revision was also observed in non-transgenic C57BL/6 mice [38].

There is experimental evidence for extrathymic origin of T-lymphocytes carrying endogenous VβTCR: i) appearance of such cells in $V\beta5^+$-transgenic mice was induced by peripherally induced tolerogen [39], was limited by lymphoid periphery (in thymus $V\beta5^+CD4^+$ T-cells remained > 95%) [40], and was not abolished by thymectomy [41]; ii) endogenous TCR-repertoire formed during revision was similar to that of non-transgenic mice [36]; iii) length of N-regions (sites of non-template nucleotide addition formed during coding segment joining) is rather short in the TCR-revised genes; this is not typical for T-lymphocytes differentiated in the thymus [36]. Nevertheless, all these data do not give a precise answer to the question whether peripheral T-cells subjected to TCR gene revision carry already formed antigen receptor.

Besides the above-discussed series of experiments in which the authors obtained maximum information from the constructed model system, there are several interesting reports on this subject available in the literature. Almost all of them also apply to $CD4^+$ T-lymphocytes. For example, rearrangement of TCRβ-locus was found in TCRαβ-transgenic mice; their lymphocytes were specific to Ac1-11-fragment of myelin basic protein (MBP). Immunization of such animals with corresponding antigen (Ac1-11) caused re-expression of RAG-1 and RAG-2 in some $CD4^+$ T-cells, induction of rearrangement followed by subsequent expression of TCR with endogenous β-chain [42].

Similar processes were also found in human peripheral blood $CD4^+$ T-lymphocytes [43]. Lantelme et al. found a population of mature T-cell with low expression of CD3. These cells spontaneously appear in the periphery, they are recognized in detectable quantities in population of mature T-lymphocytes, and their number exhibits age-dependent increase [44]. It was found that $CD4^+CD3^{low}$ T-cells express both RAG proteins, and intermediate products of rearrangement are detected in these cells: double strand breaks of DNA at RSS in TCRβ-locus [43].

Repeated rearrangement at the TCR α-locus has also been confirmed in at least two studies. The first one employed TCRα-knock-in mice. It revealed re-expression of RAG proteins in $CD4^+$ T-cells of lymph nodes, rearrangement at TCR α-locus, and substitution of transgenic α-chain for the endogenous one during *in vivo* immunization with viral superantigen [4]. The second study employed non-obese diabetic (NOD) mice prone to diabetes mellitus type I. Autoaggressive peripheral $CD4^+$ T-lymphocytes of such mice expressing membrane CD40 also exhibited induction of RAG-protein expression followed by their nuclear translocation and subsequent rearrangement at the TCR α-locus [45].

The only study providing convincing evidence for the existence of processes of secondary TCR rearrangement in $CD8^+$ T-lymphocytes was also carried out using NOD mice [5]. In NOD mice, a fraction of $CD8^+$ T-

lymphocytes associated with pancreatic islets could recognize NRP-A7 peptide. During repeated stimulation of such lymphocytes with NRP-A7-pulsed dendritic cells these lymphocytes re-expressed RAGs. This resulted in loss of NPR-A7 binding and termination of transcription of NRP-A7-specific TCR genes; instead, such lymphocytes exhibited a wide spectrum of other TCR-rearrangements. Re-expression of RAGs was also observed in $CD8^+$ T-lymphocytes of lymph nodes of both NOD-NRP-A7 transgenic and non-transgenic mice, and it could also be induced in peripheral $CD8^+$ T-cells of non-diabetic NRP-A7-transgenic mice B10.H2g7 [5].

There is a report on RAG-2 expression in $CD8^+$ T-lymphocytes of Peyer's patch. These cells expressing recombinase were differentiated in thymus as evidenced by the type of the expressed CD8 co-receptor, αβ heterodimer (in γδT-lymphocytes differentiated outside thymus CD8 molecule usually exists as αα homodimer [46]).

The table summarizes all available data on TCR rearrangement in peripheral T-lymphocytes.

The major question arises: does this phenomenon actually represent TCR revision, where cells exhibiting repeated rearrangement of TCR cells are mature T-lymphocytes with already formed antigen receptor? Only two of the seven above-cited reports directly demonstrated the presence of competent αβTCR in cells expressing recombinases [43, 46]. The authors of other reports were not interested in this problem, and they just provided some evidence for extrathymic pathways of TCR rearrangement in peripheral T-lymphocytes [36, 40, 42]. These data do not rule out the possibility that this rearrangement occurs in immature T-cells, which have left the thymus before termination of their differentiation and suppression of recombinase expression. (This might occur due to some impairments of thymic development or action of physiological factors.) This is a real scenario: analysis of coexpression of RAG-1, RAG-2, and pre-TCRα in peripheral T-lymphocytes suggests a possibility for thymocyte migration at the stage of pre-T-cells from thymus into peripheral organs and tissues, e.g., into liver [47] or gut mucosa [48, 49], and continuation of differentiation in the new environment.

Discussing rearrangement of TCR genes in immature T-cells, it is important to mention so-called receptor editing. This process plays an important role in thymic development of T-lymphocytes [50]. It is known that in contrast to the TCR β-locus, the allele exclusion by α-locus is not ultimately strict [50, 51]. The subsequent rearrangement at second allele [50, 51], resulting in simultaneous expression of two different membrane TCRαβ [52], as well as the secondary rearrangement of the same allele [51], are quite possible. The latter case involves substitution of already rearranged α-chain for a new one and formation of TCR exhibiting new antigen specificity [51, 53]. This phenomenon was defined as receptor editing. In fact, thymocytes continue α-chain rearrangement until completion of positive selection terminating recombinase selection or apoptotic death [51, 54]. Taking into consideration that the major proportion of T-cells formed in the thymus is useless (as the result of nonproductive rearrangement or inability to pass through positive selection), the second rearrangement at TCRα-locus gives possibility for maximal increase in αβ T-lymphocyte generation and the diversity of their antigen-recognizing repertoire [55]. Formally, the term "revision" is applicable for the phenomenon of antigen receptor editing because repeated rearrangement involves genes with formed TCR. However, in the modern literature these processes are separated. The term "TCR editing" is applicable for cells at intermediate stage of their development, which have not yet passed through selection. The term revision is applicable for T-lymphocytes (with presumably completed differentiation) that have left the thymus and repeatedly induced TCR gene recombination in response to various stimuli.

It is possible that rearrangement processes, recognized in peripheral T-lymphocytes, basically represent receptor edition which continues after migration of the immature T-cell to periphery. However, it should be taken into consideration that only half of all reports on this problem dealt with second rearrangement at α-locus [4, 5, 45], whereas other studies demonstrated recombination of β-chain TCR genes [3, 42, 43]. They are not involved in receptor editing, and so good evidence exists that this process involved mature T-lymphocytes rather than their T-cell precursors, so that this process may be reasonably defined as TCR revision.

The second problem, which appears irrespectively to the interpretation whether this phenomenon may be defined as revision of already formed TCR or it does represent continuation of thymic differentiation on periphery, consists in the following: if formation of new TCR occurs outside the thymus, do such T-lymphocytes undergo processes of clonal selection, providing elimination of potentially autoreactive cells. If yes, which parts of the immune system are responsible for this process? Certain evidence exists that selection processes occur in the periphery. It has been demonstrated that peripheral T-lymphocytes with endogenous TCR which are formed in transgenic mice during secondary rearrangement of TCR do not proliferate in response to lymphocytes of corresponding non-transgenic animals in combined cell culture; this means that antigen repertoire formed during TCR revision is autotolerant [3]. Such processes might be localized in germinal centers of lymphoid organs. First, the germinal centers contain $CD4^+$ (but not $CD8^+$) T-lymphocytes, and the major proportion of experimental data on secondary rearrangement of TCR genes has been obtained using $CD4^+$ T-cells [3, 4, 42, 43, 45]. In studies employing both cell subpopulations, such rearrangement was detected only in $CD4^+$, but not in $CD8^+$ T-lymphocytes [3]. Second, in the model system providing stable

Comparative analysis of secondary rearrangement of TCR genes in various experimental systems

No.	Reference	Type of T-cells subjected to TCR rearrangement	Host	Locus	Rearrangement inducer	Methods used for evaluation
1	McMahan, C.J., Fink, P.J. (1998) *Immunity* McMahan, C.J., Fink, P.J. (2000) *J. Immunol.* Ali, M., et al. (2003) *J. Immunol.* Cooper, C.J., et al. (2003) *J. Immunol.* Cooper, C.J., et al. (2004) *J. Immunol.*	$CD4^+$ T-splenocytes	TCR Vβ5-transgenic mice	β	Mtv-8 superantigen (tolerogen)	• expression of RAG-1 and RAG-2 (mRNA) • detection of rearrangement intermediate products • analysis of Vβ-antigen expression profiles
2	Lantelme, E., et al. (2000) *J. Immunol.*	$CD4^+$ peripheral blood T-cells	human (healthy volunteers)	β	—	• expression of RAG-1 and RAG-2 (mRNA) • detection of rearrangement intermediate products
3	Huang, C.-Y., et al. (2002) *J. Immunol.*	$CD4^+$ lymph node T-cells	TCRα-knock-in mice	α	Mtv-6 superantigen (tolerogen)	• expression of RAG-1 and RAG-2 (mRNA) • detection of rearrangement intermediate products
4	Serra, P., et al. (2002) *PNAS*	$CD8^+$ T-cells associated with pancreatic islets	NOD (non-obese diabetic) mice	α	chronic stimulation with NRP-A7 peptide (tolerogenic treatment)	• expression of RAG-1 and RAG-2 (mRNA, protein) • analysis of Vβ-antigen expression profiles
5	Vaitaitis, G.M., et al. (2003) *J. Immunol.*	$CD4^+$ T-splenocytes	NOD (non-obese diabetic) mice	α	CD40 cross-linking	• expression of RAG-1 and RAG-2 (mRNA, protein) • detection of rearrangement intermediate products
6	Kondo, E., et al. (2003) *Int. Immunol.*	$CD8^+$ Peyer's patch T-cells	non-transgenic mice	—	—	• expression of RAG-2 (mRNA)
7	Bynoe, M.S., et al. (2005) *PNAS*	spleen and lymph node $CD4^+$ T-cells	MBP αβ TCR-transgenic mice	β	MBP (myelin basic protein)	• expression of RAG-1 and RAG-2 (protein)

and reproducible rearrangement of TCR genes in peripheral T-cells impairments of B-lymphocyte formation or defects in CD28 expression such rearrangement was not observed [37]. (According to literature data, under such conditions germinal centers are not formed [56]). Third, germinal centers traditionally providing selective environment required for elimination of autoreactive B-cell clones [57] may theoretically maintain similar processes with respect to T-lymphocytes. Finally, recent study has demonstrated that T-lymphocytes formed during revision carry the membrane marker, CXCR5, which is typical for germinal centers; histological studies revealed the presence of T-cells exhibiting revision of TCR genes in spleen germinal centers [58]. The fact that $CD8^+$ T-lymphocytes with secondary rearrangement of TCR genes have been recognized in NOD mice predisposed to the development of autoimmune processes [5] supports the suggestion about predominant role of the germinal centers in elimination of autospecific T-cell clones, formed during TCR revision.

The other question reasonably arising during analysis of the abovementioned studies consists in mechanisms underlying induction of the rearrangement of TCR-genes in peripheral T-lymphocytes. In this connection, it is relevant to discuss an interesting report about expression of membrane CD40 in T-lymphocytes. A series of studies [45, 59, 60] revealed an unusual subpopulation of peripheral $CD4^+$ T-lymphocytes in mice predisposed to the development of autoimmune processes (e.g., in NOD mice). These lymphocytes co-expressed CD40, and $CD4^+CD40^+$ T-cells represented up to 60% of $CD4^+$ T-lymphocytes. In control BALB/c mice of the same age, such cells represented just ~5% of total number of $CD4^+$ T-lymphocytes. Moreover, development of diabetes mellitus in NOD mice was accompanied by the increase in $CD4^+CD40^+$ T-lymphocytes subpopulation [60].

Traditionally the CD40 molecule is expressed in antigen presenting cells (B-lymphocytes, macrophages, dendrite cells, etc. [61]), whereas T-lymphocytes carry its ligand, CD154, on the plasma membrane. Binding of CD40 with CD154 initiates co-stimulating signal in T-cells. This signal provides adequate lymphocyte stimulation in response to antigen. Expression of the molecule untypical for T-lymphocyte raises the question of origin and biological importance of this phenomenon. Taking into consideration that in B-lymphocytes CD40 binding leads to activation of RAG-1 and RAG-2 followed by subsequent switch of class of immunoglobulin production [62], a similar role of CD40 molecules has also been proposed for T-cells. Subsequent results confirmed this hypothesis. In the population of $CD4^+CD40^+$ T-lymphocytes, expression of both recombinases was recognized. Moreover, cross-linking of CD40 on $CD4^+CD40^+$ T-cells induced expression of RAG proteins, their nuclear translocation, and rearrangement at TCR α-locus [45, 59]. At the present time this hypothesis has at least one confirmation: using experimental model of $V\beta5^+$-transgenic mice, CD40 expression on peripheral T-lymphocytes subjected to rearrangement of TCR-genes has been recognized [58].

BIOLOGICAL IMPORTANCE OF TCR GENE REARRANGEMENT IN THE PERIPHERY

Analysis of studies on V(D)J-rearrangement in peripheral T-lymphocytes reveals modes of its induction. Usually it represents tolerogenic treatment caused by either weak interaction of T-lymphocyte with corresponding antigen [3], or certain mode of antigen administration (chronic stimulation) [5], or employment of superantigen as a stimulant [3, 4]. The two former pathways of tolerization have been known for a long time and have been confirmed in numerous studies. Interaction of superantigens with T-lymphocytes is often used as the model system for studies of extrathymic mechanisms responsible for tolerance formation. It is known that in contrast to traditional antigens, administration of superantigens to adult animals cannot induce classic immune response, accompanied by formation of immunological memory [63, 64]. As a rule, in the major proportion of superantigen-reactive cells, they induce apoptosis [65], whereas remaining cells demonstrate loss of reactivity towards the given superantigen [63, 64]. In one study on TCR revision employing the same experimental model, the superantigen (Mtv-6) induced recombinase expression and secondary rearrangement, whereas a usual antigen, cytochrome *c*, failed to induce such effects [4].

These data suggest that TCR revision represents a peripheral mechanism of tolerance formation in addition to known mechanisms such as activation-induced apoptosis, anergy (loss of activation ability in response to antigen), or antigen-specific suppression related to action of regulatory T-cells. The fact that in the experimental model of induction of peripheral tolerance to foreign histocompatibility antigen expressed outside the thymus blockade of RAG-2 expression abolished tolerization seems to indirectly support this hypothesis [66].

Moreover, it is possible that the anergy state in itself is determined by the revision of antigen receptor; at least employment of TCR revision well explains this process: a cell loses the ability for activation response to antigen because it has lost a receptor specific to given antigen and expresses a new receptor with different specificity.

The other phenomenon, which may also be directly related to TCR revision, is so-called avidity maturation of T-lymphocytes: the increase in proportion of antigen-specific T-cells with TCR of high affinity during immune response [67, 68]. In B-lymphocytes realization of this process involves at least two parallel pathways: i) at the cellular level it occurs due to changes in affinity of antigen receptor by somatic hypermutations; ii) at the population level due to preferential selection of clones with increased affinity to antigen [57]. In contrast to B-cells, the antigen

receptor of T-lymphocytes is not subjected to somatic mutations. Until recently, it was considered that avidity maturation in these cells does not involve TCR affinity and that it does occur only due to selective expansion [68] and/or due to the increase in corresponding T-cell clones life span [67]. Nevertheless, in light of new data, one may suggest that changes in antigen receptor affinity may take place; this may occur due to secondary rearrangement of TCR genes alternatively to somatic mutations in B-lymphocytes.

Peripheral TCR revision may also have negative consequences. First, formation of lymphocytes with new antigen receptor has potential danger of appearance of autospecific T-cells and, consequently, development of autoimmune processes. In this connection, it is relevant to note that secondary rearrangement has been stably detected in NOD mice predisposed to autoimmune diabetes mellitus [5, 45]. Second, reactivation of recombinases and processes of V(D)J rearrangement in mature T-lymphocytes are risk factors for development of malignant tumors in lymphoid tissues, such as lymphoma and leukemia [69]. It is likely that under normal conditions, these processes are successfully regulated and appear only under pathological conditions.

CONCLUSION

Initially the possibility of revision of antigen receptor in the periphery was discussed mainly in term of B-lymphocytes because expression of RAG proteins and rearrangement were originally demonstrated in the germinal center B-cells. This phenomenon was subsequently confirmed in numerous studies (e.g. [70-72]), and the problem of antigen receptor revision in peripheral B-cells was thought to be solved. However, in 1999 using various mouse strains three independent research groups demonstrated that recombinase expression was registered only in immature B-splenocytes recently migrated from bone marrow [34, 35, 73], and cells suppressing recombinase expression were unable to express these enzymes during immune response [35]. Subsequent studies provided reasonable explanations for these data and demonstrated that during immunization bone marrow B-cell precursors migrate to spleen more intensively, possibly due to the adjuvant induced inflammatory response [74]. According to a generally accepted concept, recombinase gene expression detected in germinal center B-lymphocytes may be defined as prolonged rather than re-induced, and it was not totally suppressed during differentiation in bone marrow. Nevertheless, induction of secondary V(D)J-rearrangement in mature B-cells cannot be ruled out, and so the question on B-lymphocyte antigen receptor revision remains open.

There is better understanding of the situation with T-lymphocytes. Registration of secondary rearrangement not only at α-locus [4, 5, 45], but also at β-locus [3, 42, 43] clearly indicates that this phenomenon cannot be solely attributed to receptor editing typical for immature T-cells because editing is ultimately related to rearrangement of α-chain only [55]. Results of some studies clearly demonstrate the presence of competent αβTCR in cells expressing recombinases [43, 46]. Peripheral V(D)J-rearrangement has been registered in various subpopulations of T-cell ($CD4^+/CD8^+$) for various genetic background (NOD, B6, B10.H-2g7, etc.). It is possible that in spite of the fact that this is a rather rare process, it does represent a normal situation rather than abnormal deviation. The fact that transgenic mice used for most of studies on TCR gene rearrangement in peripheral T-lymphocytes decreases the representation of the data obtained. However, it also gives a reasonable explanation: low frequency of precursors of antigen-reactive T-cells complicates analysis of such processes in normal animals. Nevertheless, in some studies authors were able to demonstrate processes of secondary TCR gene rearrangement not only in transgenic mice, but also in corresponding non-transgenic animals as well [5, 38].

Although all currently available data do not provide decisive evidence for the conclusion that mature T-lymphocytes, successfully terminating their thymic differentiation, undergo TCR revision, they do provide convincing evidence for the existence of peripheral TCR gene rearrangement and within this framework it is not ultimately important whether these rearrangement processes are primary or secondary ones. Usually they are induced by tolerogens irrespectively to the level of differentiation of responding cells; the rearrangement processes are accompanied by loss of initial antigen receptor and expression of membrane receptor with new antigen specificity.

According to the central dogma of T-cell development, during clonal selection T-cell precursors of T-lymphocytes effectively recognizing autoantigens are eliminated by apoptosis. This way for elimination of autospecific clones provides peripheral autotolerance, which is required for protective immune system functioning without damage to the organism's own tissues.

At first glance "disrupting" these canons, TCR revision may provide appearance of potentially autoreactive peripheral T-cells. However, this is not a large-scale phenomenon, and TCR revision is not the only process which may alter natural autotolerance formed in thymus [75, 76]. Under pathological conditions, TCR revision may contribute to persistence and/or progression of chronic autoimmune diseases as it takes place in NOD mice [5, 45]. However, under normal conditions these processes are controlled and do not have marked manifestations [77]. Recent data also indicate that TCR revision occurs in the germinal centers [58], and these centers have a cell microenvironment that is responsible for effective elimination of autospecific clones of at least B-

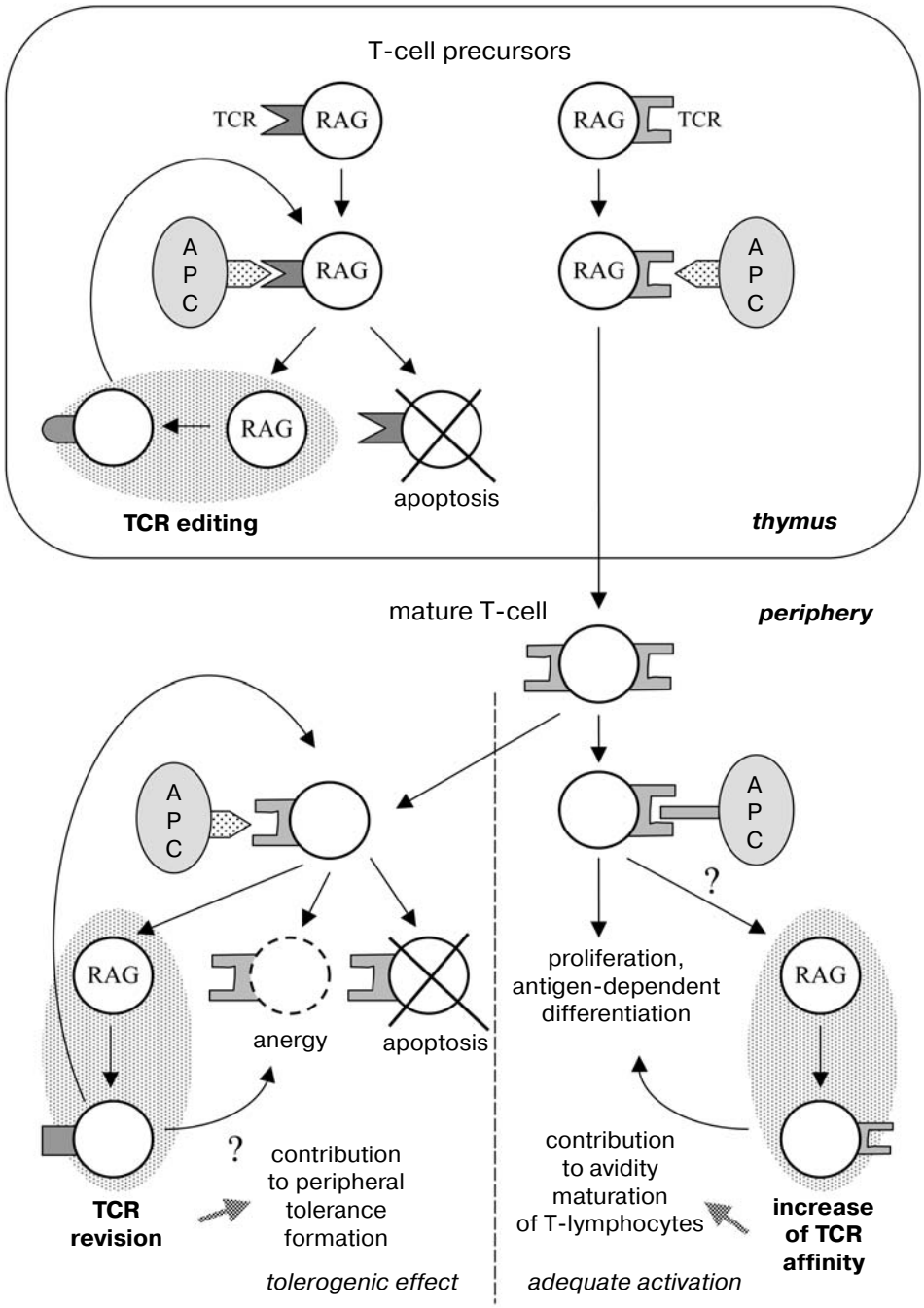

Fig. 2. Potential role of antigen receptor revision in the development and functioning of T-lymphocytes (see explanations in the text). APC is antigen-presenting cell.

lymphocytes [57, 77]. It is possible that such scenario is also applicable for T-cells. In this connection it is important to pay attention to the report that antigen-recognizing repertoire of T-lymphocytes formed during the second rearrangement is autotolerant [3]. If this study gets further an independent support using other experimental models, the problem of TCR revision as a putative risk factor for the development of autoimmune diseases will be abolished.

Secondary rearrangement of TCR genes plays a positive role in the immune system functioning because it is involved in tolerance formation. At the stage of thymic development, it represents a mode of receptor editing, which is involved in formation of central tolerance. At subsequent stages, this process represents one of the mechanisms responsible for induction of peripheral tolerance by inducing changes in T-cell antigen receptor specificity in response to stimulation (Fig. 2). TCR revi-

sion also represents the additional mode for formation of antigen-recognizing repertoire diversity. It may be involved in avidity maturation of T-lymphocytes during immune response or may represent an effective tool of immune system functioning against viral or microbial mutants appeared during infection. It may also form a reserved pool of T-lymphocytes specific to antigens that do not have corresponding TCR in the host organism.

In general, TCR revision should give certain advantages to host organisms provided that effective mechanisms for removal of autospecific T-cell clones inevitably appearing in the process of secondary TCR gene rearrangement exist.

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