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PERSPECTIVE



Total threshold cytotoxicity of therapeutic antibodies for selective destruction of pathogenic memory T cells: implications for immunotherapy of autoimmune and allergenic disorders

Victor I. Seledtsov^a and Galina V. Seledtsova^b

^aCenter for Medical Biotechnologies, Immanuel Kant Baltic Federal University, Kaliningrad, Russia; ^bLaboratory for Cellular Technologies, Institute for Fundamental and Clinical Immunology, Novosibirsk, Russia

ABSTRACT

Introduction: Pathogenic memory CD4⁺ T cells are the mainspring of autoimmune and allergic disorders, suggesting that effective pathogenetic immunotherapy should be primarily directed onto their direct inactivation without affecting normal cells.

Areas covered: A novel immunotherapeutic concept is proposed that applies suboptimal doses of several cytotoxic antibodies (Abs) against membrane antigens (Ags) (such as CD4, CD45RO, CD69, CD103, CD27, CD38, DR, etc.) with a view to achieve a threshold density of immune complexes on pathogenic memory CD4⁺ T cells for their selective elimination. During disease exacerbations, a complex Ab formulation could be applied to combine Abs against CD4, CD45RO, and CD69 to selectively destroy both activated memory CD4⁺ T cells located in lymphoid tissues and resident memory CD4⁺ T cells present in local inflammatory sites in situ. In contrast, normal T cells are spared from destruction as being recognized only by some Abs leading to Ab-Ag complexes below cytolytic threshold levels. Inactivation of pathogenic CD4⁺ T cells will also withdraw T helper support to pathogenic memory B cells and memory CD8⁺ T cells, thus effectively diminishing their activity.

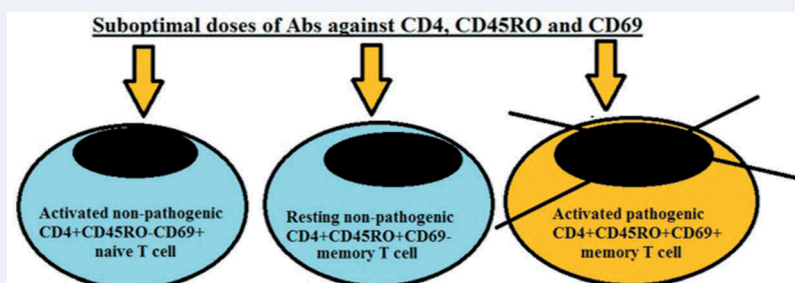
Expert opinion: The described approach benefits from universality and potential availability of a vast library of monoclonal Abs with relevant specificity.

ARTICLE HISTORY

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KEYWORDS

Therapeutic antibody; antibody-mediated cytotoxicity; immune complex; pathogenic memory T cell; autoimmune disease; allergy



The presence of Abs against CD4, CD45RO and CD69 in suboptimal concentrations in cell microenvironment would fail to result in achieving critical threshold cytolytic density of membrane-associated Ag-Ab complexes on activated CD4⁺CD45RO⁻CD69⁺ naive (non-pathogenic) T cells involved in protective immunity, as well as on resting CD4⁺CD45RO⁺CD69⁻ memory T cells not implicated in pathological immunogenesis. On the contrary, this formulation would be capable of achieving cytolytic threshold density of Ag-Ab immune complexes on the surface of CD4⁺CD45RO⁺CD69⁺ central memory, effector memory and tissue-resident memory T cells activated during disease exacerbation and involved in disease immunopathogenesis. Thus, the proposed formulation of therapeutic Abs could cause a rapid positive clinical response due to selective inactivation of pathogenic helper CD4⁺ T cells and to reduction of functional activity of pathogenic CD8⁺ T cells and B cells which are known to be dependent on helper CD4⁺ T cells.

1. Introduction

Acquired immunity plays a major role in facilitating antigen (Ag) recognition and the development of faster and more effective immune responses upon secondary Ag encounter. Mature T and B lymphocytes are known to be central cellular mediators of specific immunity. Upon infection or vaccination, complex antigenic structures are cleaved to antigenic peptides inside Ag-

presenting cells (APC) for subsequent presentation in the context with major histocompatibility complex (MHC) molecules on the surface of APC to T cells. Cognate Ag recognition triggers T-cell receptor (TCR)-mediated signaling, which collaborates with various other signals (such as of cytokine, co-stimulatory, chemokine, integrin, and metabolic nature) to facilitate the generation of memory T cells of different phenotype and function. In contrast to TCRs, B-cell receptors (BCRs) recognize intact Ags without the

Article Highlights

- Pathogenic memory CD4⁺ T cells are the mainspring of autoimmune and allergic disorders.
- Simultaneous administration of suboptimal doses of cytotoxic antibodies against CD4, CD45RO, and CD69 molecules could selectively destroy pathogenic memory helper T cells with minimal side effects in clinical settings.
- Inactivation of pathogenic CD4⁺ T cells could withdraw T helper support to pathogenic B cells and CD8⁺ T cells, thus effectively eliminating other pathogenic mechanisms.
- A novel concept is proposed, which can potentially use a plethora of available Ags against surface markers (such as CD103, CD27, CD38, DR, etc.) expressed on pathogenic T lymphocytes as targets for therapeutic Abs in order to attain critical cytotoxicity levels necessary for selective elimination of pathogenic T cells.

need for Ag processing and presentation of antigenic peptides by MHC molecules. Importantly, B-cell-dependent immunological phenomena, such as B-cell differentiation and memory formation are reliant on continuous help from Ag-reactive T cells, such as that provided by co-stimulatory molecules and cytokines [1,2]. It has been shown that upon secondary Ag encounter, memory T cells induce faster and more pronounced pathogen-specific immune responses. However, the immunological memory is not without certain disadvantages, particularly as far as the development of autoimmune diseases is concerned. Indeed, in the course of immune responses in addition to pathogen-specific memory T cells, self-reactive memory T cells are generated, constituting the foundation for the development of a highly efficient pathogenic and cytotoxic immunity against self-Ags and tissues [3]. Similarly, in case of allergic conditions, allergen-specific memory T cells are generated and promote downstream exaggerated pathogenic tissue-destructive immunity against relevant exogenous Ag (allergen) [4]. Obviously, pathogenetic therapy of both autoimmune and allergic disorders should be primarily directed onto inactivation of pathogenic memory T cells. However, all therapeutic modalities for treatment of inflammatory immunological disorders available today do not resolve this conundrum in a satisfactory way. The main disadvantage of these drugs consists in the absence of selectivity of their immunosuppressive activity, such that both noxious and innocuous immune reactivity is equally affected. In addition, immunosuppressant drugs could also cause damage to healthy tissues and organs of the body. In this paper, we outline a fundamentally novel approach to achieving selective inactivation of pathogenic T cells in the clinics, based on reaching threshold cytotoxicity of humanized antibodies (Abs) administered in suboptimal concentrations. As such, our paper also provides first theoretical conceptualization of a recently emerged approach attempting application of two or more therapeutic Abs with different Ag specificity in experimental anti-cancer immunotherapy [5,6]. The importance of the proposed theoretical concept for developing novel immunotherapeutic modalities against both autoimmune and allergic diseases cannot be overestimated due to its universality and potential availability of a vast library of monoclonal Abs with relevant specificity. This was the primary reason that prompted us to write this paper with a view to spark new practical laboratory and clinical research along the lines proposed. Here, we performed an extensive literature

data mining on the topics of theoretical immunochemical mechanisms underlying threshold cytotoxicity, memory T cells, antibody-mediated cytotoxicity, and other relevant subjects. We analyzed data obtained from PubMed, immunology and immunochemistry handbooks, and other open sources and scrutinized the concept against our solid laboratory and clinical expertise in immunotherapeutic drug R&D.

2. Memory T-cells and their role in pathogenesis of immune disorders

Upon Ag exposure, conventional naive T cells can differentiate into effector T cells (Teffs), effector memory T cells (TEMs), and central memory T cells (TCMs). In addition, long-lived memory T cells are derived from T cell clones carrying particular TCRs that recognize Ags in a most effective manner; on secondary Ag exposure long-lived memory T cells help mount quicker and stronger Ag-specific immune responses [1,2]. Generally, memory T cells are characterized by CD45RO⁺ and CD45RA⁻ phenotype, whereas naive T cells express CD45RA, but not CD45RO. In addition, memory T cells exhibit enhanced expression of CD2, CD11a, and CD44 markers, as compared to naive T cells [7]. It is recognized that central and effector memory T cells are less dependent on the contact with MHC-peptide complexes for survival, compared with naive T cells. In addition, TEM pool is capable of self-renewing 3–4 times faster than naive T cell subset [8]. TCMs have been described to possess: (i) CCR7⁺ CD62L^{hi} phenotype, (ii) ability to circulate to secondary lymphoid tissues, (iii) greater proliferative capacity, and (iv) extended lifespan. In addition, TCMs are believed to be important for production of Teffs following Ag recall. TEMs are known to express CCR7 and CD62L^{lo} markers, which are essential for their circulation to peripheral non-lymphoid tissues and endow them with immediate effector functions [3,9]. Compared to TCMs, Teffs are thought to be: (i) more short-lived, (ii) have lower proliferate capacity, and (iii) of greater cytokine production potential [9]. A novel class of memory T cells, namely tissue-resident memory T cells (TRMs) has been recently described, which lack CCR7 expression (similar to TEMs), but do express an early T-cell activation marker CD69 (human transmembrane C-Type lectin protein), which appears to be critical for the development and retention of TRMs *in situ* [3,7]. Indeed, human TRMs reside permanently in the peripheral tissues and have tissue-specific properties, such that, for example, CD4⁺CCR7⁻CD69⁺ TRMs are the predominant CD4⁺ T cell subset in the lungs, intestines, skin and bone marrow [9]. In general, entry and retention of TRMs into tissues is controlled by the expression of various markers. Thus, TRMs residing in the skin express cutaneous lymphocyte molecule (CLA, a glycovariant of P-selectin glycoprotein ligand-1 (PSGL-1)), which grants them access to the skin and skin-homing chemokine receptors (CCR4 and CCR10); TRMs in small intestine and colon express a gut-homing receptor CCR9 and $\alpha\beta 7$ integrin; and TRMs in the lungs up-regulate CCR6 expression. Altogether, there is mounting evidence that TRMs play a role in immunoprotection and immunopathology, thus constituting a viable target for immunotherapeutic interventions [9].

Memory T cells have been shown to be a pivotal factor in pathogenesis of various autoimmune diseases, such as

multiple sclerosis (MS), rheumatoid arthritis, type 1 diabetes, and psoriasis. Thus, higher percentages of memory T cells (similar to the levels characteristic of healthy 20–30-year-old adults) were demonstrated in pediatric MS patients compared to healthy age-matched control group. Adult MS patients had also increased frequencies of memory T cells, especially TEMs, which appear to be homing specifically to the cerebrospinal fluid and parenchymal MS lesions triggering local autoimmune inflammation [7]. Indeed, upon activation encephalitogenic CD4⁺ Th1 T cells have been shown to produce pro-inflammatory cytokines, such as interferon (IFN)-gamma, tumor necrosis factor (TNF)-alpha, interleukin (IL)-2, IL-17, and granulocyte-macrophage colony-stimulating factor (GM-CSF), recruit macrophages, CD8⁺ T-cells, and NK cells thus launching cell-destructive processes in nervous tissues [10,11]. In addition, these T-cells provide help for mounting B-cell responses and production of myelin-specific Abs that in turn further enhance tissue destruction via both Fc receptor- and complement-mediated mechanisms [11,12]. Pathogenic changes in proportion and functional activity of memory T cells in peripheral blood were demonstrated in other clinical conditions and target tissues [7]. In support of this notion, human TRMs have been postulated to contribute to brain injury in MS and schizophrenia, and pathogenic autoreactive TRMs were found to induce fixed recurrent skin lesions in patients with psoriasis. Those lesions tended to be well demarcated and showed distinct margins and an abrupt cutoff with normal tissues, supporting a well-known fact that TRMs are a non-recirculating highly compartmentalized T cell subset [4]. Indeed, in patients with lupus erythematosus and nephritis individual T cell clones in renal inflammatory infiltrates persisted for years in repeat biopsy samples from individual patients, suggesting also that TRMs may participate in the progressive devastating kidney damage observed in patients with lupus [4].

T cell memory-mediated immune responses could be also potentially harmful when directed against allergens. Most allergic disorders are characterized by Th2-type immune reactivity, such that during the sensitization phase allergen-specific CD4⁺ Th2 cells have been described to expand and acquire type 2 cytokines production capacity, followed by up-regulation of chemokine receptors and integrins associated with migration to various anatomical sites [13]. Allergic diseases, such as asthma and other allergic airway diseases, food allergies and celiac disease are usually characterized by rapid inflammation onset. The occurrence of allergic inflammation within 24–48 h upon Ag exposure could indicate the involvement of TRMs into the pathogenesis of the allergic disease. In these settings, non-circulating Th2 TRMs produced IL-4, IL-13 and large quantities of IL-5, accumulated at local sites of chronic inflammation and induced allergic eosinophilic inflammation [13].

Lately, several avenues of research have been pursued aiming to target pathogenic T cells and introduce novel immunotherapeutic modalities and approaches into clinical practice. One notable example refers to agents blocking co-stimulation pathways, which have demonstrated limited success possibly due to the fact that memory T cells are less dependent on co-stimulation, as compared to naive T cells [3]. Another example

is regulatory T-cell (Treg) infusion therapies, which potentially held great promise in terms of providing a tool to control adverse immune responses. However, there is evidence that memory T cells are relatively more resistant to Treg-based suppression immunotherapies, as compared to naive and effector T cell subsets [3], which could make this approach less effective with respect to selective inactivation of pathogenic memory T cells. T-cell depletion therapy was found previously to work, at least preventatively, in most experimental arthritis models, but essentially failed in human rheumatoid arthritis settings [14]. In the similar vein, total T-cell depletion in the absence of specific measures to ensure selective TEM removal and retention of Tregs was considered as an ineffective treatment for type 1 diabetes [15]. Unfortunately, detrimental serious side effects of such unselective T-cell depletion tended to exceed the clinical benefits of such treatment. Depletion of CD4⁺ T cells also did not show clinical efficacy as far as treatment of chronic autoimmune diseases is concerned [16].

Taken together, CD4⁺ T cells can be considered as key mediators of both protective and pathogenic immune reactivity, with phenotypical immunological marker analysis across various technological platforms allowing for clear identification of appropriate T cells and their activation status. Based on this solid technological foundation and recent advances in fundamental immunology, here we proposed a novel immunotherapeutic platform for treating immunological disorders, which uses a formulation of humanized Abs against various membrane-associated molecules expressed on the surface of memory CD4⁺ T cells. We envisage that concurrent immunotherapeutic application of these Ab-based drugs in suboptimal dosages would ensure high selectivity of pathogenic T cell destruction in patients with autoimmune and allergic disorders.

3. Antibody-mediated cytotoxicity and its possible application for selective destruction of pathogenic T-cells

Ab-mediated cytotoxicity is known to be implemented via three major fundamental immune mechanisms, and namely complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC), and complement-dependent cellular cytotoxicity (CDCC). CDC is triggered upon IgM or IgG binding to the cell surface, which sets off a signaling cascade consisting of more than 30 proteins leading to membrane-attack complex formation and cell lysis. Binding effector cells (such as granulocytes, macrophages, and NK-cells) to target cells coated with specific Abs are known to initiate a downstream cytolytic process called ADCC [17]. Activation of complement and deposition of C1q, C3b, iC3b or C4b complement components on target cells results in cell destruction via CDCC pathway. In this respect, Abs that facilitate deposition and activation of complement components on the cell surface are of primary importance in triggering complement-dependent cell-mediated cytotoxicity [17–19]. Taken together, interactions of various ligands described above with appropriate receptors expressed on granulocytes, macrophages, and NK-cells are necessary and sufficient to induce Ab-mediated cytotoxic activity. From the

immunochemistry point of view, Ab-mediated cytotoxic effect is described by a threshold phenomenon, which postulates that membrane-associated immune complexes have to reach certain threshold density to be able to activate cytolytic process, which was experimentally confirmed with both polyclonal [20–24] and monoclonal Abs [25–28] specific for various membrane-associated Ags. While density of cell membrane-associated immune complexes is defined primarily by membrane Ag expression level and Ag-specific Ab concentration, other factors are also at play, such as affinity of Ag-Ab interactions, and stability of binding of immune complexes to the cell membrane [29]. In addition, IgG isotype affects the efficacy of cell lysis, and IgG1/IgG3 are known as potent activators of the classical pathway of human complement. The majority of approved recombinant monoclonal Ab therapies are of the human IgG1 subclass, which can engage both humoral and cellular components of the immune system. There are no approved IgG3 therapeutics due in part to the relatively short half-life of IgG3 [30]. Taken together, Ab-mediated cytotoxicity occurs when a large number of high-affinity Ag-specific Abs effectively interact with appropriate Ags abundantly expressed on the cell surface. We envisage that critical cytotoxic threshold could vary to some extent depending on the particular cell type and expression of membrane molecules, which could potentially suppress Ab-dependent cytotoxicity (such as KIRs). The threshold phenomenon offers itself as an important mechanism that controls excessive cell cytotoxicity, which could occur due to minor innocuous antigenic challenges constantly present in the environment. Based on these considerations, we envisage two disequilibrium scenarios when Ab-mediated cytolytic process could not be launched: (i) when high (optimal) Ag-specific Ab concentrations are present along with low (suboptimal) membrane-associated Ag expression levels, and (ii) *vice versa*, high membrane-associated Ag expression levels co-exist with low (suboptimal) Ag-specific Ab concentrations. Certainly, other factors such as instability of immune complexes would add to the complexity of regulatory mechanisms that control Ab-mediated cytotoxicity.

Major progress has been achieved in a different immunotherapeutic niche of low-immunogenic humanized and

fully human Abs, which greatly benefited from modern bioengineering allowing for manufacturing low-immunogenic cytotoxic IgG Abs of nearly any specificity. Several anti-cancer Abs-based modalities of this kind have been recently introduced into clinical practice, and therapeutic Abs have become an essential treatment strategy for a variety of disorders, including autoimmune and allergic diseases in general [17,31]. Based on these and other advances, a novel immunotherapeutic concept could be proposed, which involves application of more than one membrane Ag-specific Ab in suboptimal dosages in order to achieve selective destruction of pathogenic immune cells. Briefly, upon Ab administration, the ensuing Ag-Ab complexes formed on the cell surface would reach a threshold cytolytic density only on pathogenic cells if and when recognized by all Abs included in the immunotherapeutic Ab formulation. In contrast, non-pathogenic immune cells would be recognized by some (but not all) Abs, thus effectively keeping membrane-associated Ag-Ab complexes below the critical cytotoxic threshold level, without triggering cytotoxicity. Specifically, application of suboptimal dosages of Abs against CD4, CD45RO, and CD69 could be efficient in selective destruction of pathogenic CD4⁺ memory T cells. Figure 1(a,b) illustrates the notion that presence of such Abs in suboptimal doses in cell microenvironment would fail to result in achieving critical threshold cytolytic density of membrane-associated Ag-Ab complexes on activated, non-pathogenic CD4⁺CD45RO[−]CD69⁺ naïve T cells involved in protective immunity, as well as on resting CD4⁺CD45RO⁺CD69[−] memory T cells not implicated in pathological immunogenesis. We argue that the above Ab formulation would not affect protective immunity mediated via CD8⁺ T cells and B cells. On the contrary, this formulation would be capable of achieving cytolytic threshold density of Ag-Ab immune complexes on the surface of CD4⁺CD45RO⁺CD69⁺ central memory and effector memory T cells activated during disease exacerbation and involved in disease immunopathogenesis (Figure 1(c)). Since activated CD4⁺CD45RO⁺ TRMs are known to express CD69 in a stable fashion, these cells will be also targeted for Ab-mediated cytotoxicity. Published data suggest a critical role of CD69⁺ TRMs in both autoimmune [32] and allergic

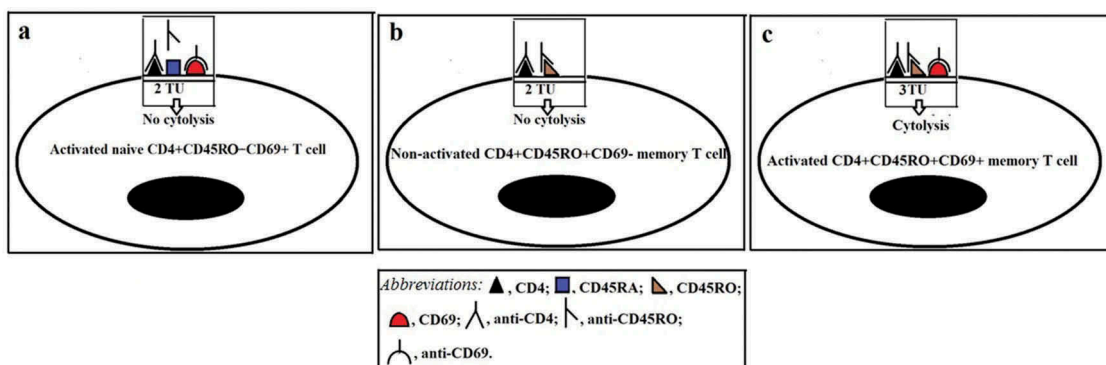


Figure 1. (a–c) Selective destruction of a pathogenic CD4⁺ memory T cell due combined activity of anti-CD4, anti-CD45RO, and anti-CD69 therapeutic Ab-based drugs. The scenario shown here assumes that 3 tentative units (TU) constitute a critical threshold cytolytic density of membrane-associated Ag-Ab complexes. When administered simultaneously in suboptimal doses, anti-CD4, anti-CD45RO, and anti-CD69 achieve immune complex density of 2 TU (i.e. below the threshold) on the surface of an activated naïve CD4⁺CD45RO[−]CD69⁺ T cell (a), as well as on the surface of a non-activated memory CD4⁺CD45RO⁺CD69[−] T cell (b), with no cytotoxicity occurring. Meanwhile, these Abs acquire the cytolytic threshold level (3 TU) of Ag-Ab immune complexes on an activated memory CD4⁺CD45RO⁺CD69⁺ T cell, followed by its lysis (c).

inflammation [33]. Consistent with our concept, anti-CD69 antibody treatment has been recently reported to inhibit an established airway inflammation in a mouse model of asthma [34].

We envisage that application of the proposed Ab preparations will not affect significantly Treg cell subset, as these cells (most of them) do not express CD45RO. Moreover, we propose using this approach in acute phases and disease exacerbation episodes, when activated pathogenic T cells would dominate over regulatory T cells and minimize immunosuppressive effects of the latter. Noteworthy, local pathological inflammation could improve the availability of pathogenic lymphocytes for the action of therapeutic Abs.

The proposed formulation of therapeutic Abs could cause a rapid positive clinical response due to inactivation of pathogenic helper CD4⁺ T cells, which, in turn, would inevitably reduce functional activity of pathogenic CD8⁺T cells and B cells known to be dependent on helper CD4⁺ T cells.

4. Expert opinion

There are several practical challenges related to the combinatorial Ab approach, such as dosages of Abs to be used in individual patients, T-cell depletion monitoring, as well as effects on other T cell subsets, such as Tregs, just to name a few. Routine monitoring of T cell subsets before and after administration of single/multiple Ab-based drugs may prove to be a useful strategy for assessing depletion efficacy of the particular T cell population in the periphery, as well as bystander effects on other T cell subsets. There have been several technological platforms developed recently that allow for determination of threshold (and sub-threshold) Ab doses in various experimental and translational settings [35,36], which could be applied for establishing particular dosages of Ab-based drugs for individual patients in the context of the proposed paradigm. We hypothesize that complex Ab preparation would be characterized by a certain therapeutic range, as is the case with other drugs, with doses of individual Abs having been preliminarily established in order to ensure maximal clinical effects (i.e. destruction of pathogenic lymphocytes) and minimal side effects (i.e. preservation of non-pathogenic protective lymphocytes).

Clearly, any Ab formulation designed for selective elimination of particular immune cells should be standardized and applied in controlled clinical settings in a specialized facility taking into account weight and body surface area of the patient. In addition, in accordance with procedures applied for other preparations, individual dosing schedules for an Ab drug would be adjusted with regard to disease severity and disease state. Clearly, more time and effort will be necessary for precise dosing of the proposed therapy, but higher specificity of T cell-depletion and absence of serious side effects could greatly outweigh these technical considerations.

We stress that mechanisms underlying Ab-mediated cytotoxicity are controlled by multiple membrane-associated and soluble immune factors, such that immune cell dysfunctions, complement depletion, and IgG-containing immune complexes present in patients' serum can reduce Ab-mediated cytotoxicity [2]. Therefore, passive Ab-based immunotherapy regimens have to be highly personalized and supplemented with comprehensive

set of supporting clinical measures (such as transfusion of donor leukocytes and/or of fresh complement-sufficient plasma preparations) aiming to maximize clinical effectiveness of the immunotherapeutic approach proposed here. In summary, we believe that further practical efforts are necessary to develop our novel combinatorial monoclonal Ab approach allowing for attaining threshold cytotoxicity levels for targeted elimination of pathogenic T cells in autoimmunity and allergic settings.

5. Conclusion

The technology discussed here does not restrict the spectrum of membrane-associated Ags that could be exploited as targets for therapeutic Abs. Theoretically, membrane-associated Ags or structures suitable for the Ab threshold-based therapy are likely to meet the following requirements: (i) to the extent possible, their distribution should be restricted to the immune cells, (ii) their expression should be stable, and (iii) they should not function as receptors for endogenous soluble ligands (otherwise this ligand and therapeutic Ab would be in an antagonistic relationship). Apart from the membrane-associated markers mentioned above, CD103, CD27, CD28, CD38, DR, and other molecules associated with functional characteristics and activation status of T cells could potentially comply with the outlined criteria. The technology proposed here can accommodate two and more Ab species specific for defined membrane-associated Ags, but the more Abs are used the lower dosages for each individual Ab should be applied. This approach will decrease risks of developing serious complications resulting from any single Ab administered at the optimal therapeutic dose. From the above-described concept, it also appears that therapeutic Abs conjugated with artificial cytotoxic molecules could be also applied for selective T-cell destruction. The main requirement for such molecules should be the existence of an achievable quantitative threshold for triggering their cytotoxic action.

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Declaration of interest

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