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Killer artificial antigen-presenting cells: the synthetic embodiment of a ‘guided missile’

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Abstract

At present, the treatment of T-cell-dependent autoimmune diseases relies exclusively on strategies leading to nonspecific suppression of the immune systems causing a substantial reduced ability to control concomitant infections or malignancies. Furthermore, long-term treatment with most drugs is accompanied by several serious adverse effects and does not consequently result in cure of the primary immunological malfunction. By contrast, antigen-specific immunotherapy offers the potential to achieve the highest therapeutic efficiency in accordance with minimal adverse effects. Therefore, several studies have been performed utilizing antigen-presenting cells specifically engineered to deplete allo- or antigen-specific T cells (‘guided missiles’). Many of these strategies take advantage of the Fas/Fas ligand signaling pathway to efficiently induce antigen-presenting cell-mediated apoptosis in targeted T cells. In this article, we discuss the advantages and shortcomings of a novel non-cell-based ‘killer artificial antigen-presenting cell’ strategy, developed to overcome obstacles related to current cell-based approaches for the treatment of T-cell-mediated autoimmunity.

Keywords

antigen-presenting cell; apoptosis; artificial; killer artificial antigen-presenting cell; T cell

Approximately 5% of the Western population are suffering from autoimmune diseases. Those individuals are in need of a long-term treatment to prevent organ damage and to reduce disease-related mortality. Still, the most common treatments of autoimmune diseases rely on corticosteroids as well as immunosuppressive drugs such as purine analogs, alkylating agents and calcineurin inhibitors [1]. Even though the use of such immuno suppressive drugs has greatly improved the prognosis of patients suffering from autoimmune diseases, these treatments do not provide a cure for the underlying immunological malfunction, and the ability to control concomitant infections or malignancies are frequently impaired. Furthermore, treatment does not result in prolonged periods of drug-free remissions.

Recently, new biological agents including monoclonal antibodies (mAbs) have been introduced, targeting inflammatory effector molecules or defined proteins and receptors expressed on the surface of pathogenic immune cells. Promising results have been observed

in several autoimmune diseases including systemic lupus erythematosus, rheumatoid arthritis (RA) and multiple sclerosis by utilizing mAbs against CD20, leading to an efficient B-cell depletion with an excellent safety record at the same time (reviewed in [2]). Anti-TNF mAb and soluble recombinant TNF receptor–immunoglobulin (Ig) fusion proteins have been demonstrated to suppress inflammation and improve function in patients with RA efficiently [3]. In the meantime, numerous mAbs or constructs targeted towards cytokines, receptors, integrins and chemokines have been recently approved or are currently under investigation in clinical trials. But all these strategies are not exclusively restricted to pathogenetically relevant cells or mechanisms, leading to potentially severe side effects [4–6]. Therefore, there is still a necessity for the development of novel selective antigen-specific immunotherapies to further improve the treatment outcomes with reduced adverse effects at the same time.

The idea of an antigen-specific therapy dates back to the 1970s and 1980s, when several groups discovered the existence of naturally occurring antigen-presenting cells (APCs) that suppressed peripheral T cells in an antigen-specific fashion. Such ‘veto cells’ presented autoantigens on their cell surface, facilitating elimination of T cells recognizing such antigens (reviewed in [7]). These findings were essentially amplified in the late 1980s and early 1990s through the discovery that natural APCs can be artificially modified to become ‘artificial veto cells’. These cells were decorated with specific protein constructs, such as HLA-A2, to modulate the antigenic display; costimulators such as CD80, CD86 and cytokines such as IL-2 via decay-accelerating factor-mediated binding of a glycosylinositol phospholipid moiety. The artificial veto cell concept was based on the enforced expression of ‘co-inhibitors’ such as CD8, which induces inhibition of T cells upon antigenic recognition (reviewed in [8]).

Based on these initial findings, it has been assumed that *trans*-signaling molecules known to induce apoptosis or programmed cell death, such as Fas ligand (FasL) and TNF, or the overexpression of immunosuppressive molecules including IL-10, TGF- β and cytotoxic T-lymphocyte antigen 4 would facilitate the therapeutic use of artificial veto cells, leading to the concept of a ‘killer APC’ or ‘guided missile’ (reviewed in [9]). Over the years, killer APCs and guided missiles were reinvented several times and in multiple facets, always aiming for an antigen-specific suppression of auto- or allo-reactive T-cell responses. Thus, dendritic cells (DCs) emerged as an attractive target owing to their pivotal potency in uptaking, processing and presenting foreign and self-antigens to T cells (reviewed in [10]).

The major advantage of the killer APC or guided missile concept compared with conventional treatment strategies originates in an antigen-specific elimination or suppression of disease-causing T cells without restraining the immune response towards viral, bacterial or tumor antigens. Furthermore, there is a reasonable possibility that treatment with such guided missiles *in vivo* will have limited side effects.

This article will focus on the development of an artificial guided missile-derived novel strategy utilizing the Fas/FasL signaling pathway to deplete autoreactive T cells in an antigen-specific manner. We will provide an overview of cell-based and artificial concepts that finally led to the development of a killer artificial APC (KaAPC).

Cell-based depletion strategies

Prerequisite to all these concepts is the tight interaction of T cells with professional APC. If only a T-cell receptor–HLA interaction occurs without further stimulation, functional anergy is induced in responder T cells (Figure 1A). However, the concomitant presentation of a prominent costimulatory signal leads to efficient activation and induction of clonal expansion in T cells (Figure 1B). Besides these two signals, killer APCs provide an additional apoptosis-inducing ‘third signal’ mediated through death-inducing *trans*-signaling molecules such as FasL. Thus killer APCs induces antigen-specific apoptosis in activated Fas⁺ T cells, whereas

FasL expression can be achieved in APCs utilizing gene transduction or exogenous loading with Fas-activating molecules (Figure 1C).

To date, FasL-expressing APCs derived from B-cell lines [11], macrophages [12] and DCs [13] have been successfully utilized for the generation of killer APCs in different *in vitro* and *in vivo* animal models resembling features of RA [14], allogeneic transplantation [15–18], allergy [19] and animal models used for the analysis of antigen-specific T-cell responses [11, 12, 20–22]. Thus, substantial suppression of allo- and auto-antigen-specific T-cell responses has been demonstrated. However, Fas is also expressed within and on the surface of these engineered APCs, rendering these cells susceptible for Fas/FasL-mediated autocrine or bystander apoptosis. To prevent this limitation, killer APCs have been derived from precursor cells with established defects in the Fas/FasL signaling pathway [13, 16, 23], Fas⁻ cell lines [16, 17, 20, 21] or cell lines with a defective Fas/FasL signaling pathway [11, 24].

By contrast, studies performed with primary human cells are still limited. Hoves *et al.* could generate murine FasL-expressing human primary mature DCs that induced apoptosis in primary allogeneic Fas⁺ T cells, and at the same time failed to deplete primary naive T cells [25, 26]. In addition, FasL-transduced human cell lines have been used to achieve alloantigen-specific T-cell tolerance and preservation of protective antiviral T-cell responses at the same time [27, 28]. However, studies demonstrating antigen-specific suppression of T-cell responses utilizing human FasL-expressing killer APCs are still missing.

Besides these promising results that envisage FasL-expressing killer APCs as a potent therapeutic strategy for the treatment of allograft rejection [17, 18, 29], autoimmune disease [27, 30–34] and chronic infections [24, 35], there are several restrictions that clearly limit their application: isolation, enrichment and differentiation of APCs are time, cost and labor intensive. Multiple enrichment cycles from peripheral blood mononuclear cells are required; however, many patients exhibit cytopenia due to chemotherapy or immuno suppressive drugs. Therefore, the amount of cells is strongly limited and the quality highly variable. Hence, it is almost impossible to assure a defined reproducible immunosuppressive APC phenotype that can be potentially applied for the modulation of the immune response *in vivo*. Furthermore, abnormal regulation of the immune system is the major cause for the development of autoimmune diseases. Therefore, the utilization of FasL-expressing killer APCs with varying phenotypes might lead to a potential unknown hazard and increased risk of further immunological malfunction. Moreover, generation of several FasL-expressing killer APCs requires viral transduction. Consequently, an inefficient transduction of APCs would increase the risk of a secondary immune response towards viral vector antigens initiated by nonfunctional killer APCs. On the other hand, a too exalted FasL expression could result in loss of a protective immune response via unspecific bystander depletion of vector-specific T cells. In addition, even if a stable FasL expression could be achieved, FasL can be cleaved from the cell surface through a metalloproteinase-dependent mechanism, resulting in a possible inhibition or unspecific activation of the Fas/FasL signaling pathway by soluble FasL, respectively (reviewed in [36]). Therefore, studies have been performed utilizing a genetically modified FasL missing the metalloproteinase cleavage side [37]. Furthermore, killer APCs are sensitive to cytotoxic effector functions of T cells, resulting in their elimination and consequently diminished killing efficiency. Hence, the therapeutic outcome and the consequences for the immune system seem unpredictable, if utilizing killer APCs [38].

Therefore, the crucial factor for a successful antigen-specific treatment is a defined and robust phenotype of FasL-expressing killer APCs, which appears unfeasible to achieve given the currently available viral and nonviral transduction methods for cellular approaches.

Novel artificial concepts paving the way to KaAPC

The development of the MHC tetramer technology has established many new possibilities of immune modulatory strategies [39]. Tetramers not only allow for visualization of antigen-specific T cells but also permit investigation of TCR and MHC interactions [40]. Previously, it has been demonstrated that H-2K^b dimer molecules bind to H-2K^b alloreactive T-cell clones and primary T cells in nanomolar concentrations, thereby inhibiting the cytotoxic effector functions of those T cells [41]. Further evidence for an inhibitory influence of MHC class I-tetramers was derived from experiments with female B6 mice. Multiple injections of HY-D^b-tetramer prevented a graft rejection of male B6 skin transplants. These results emphasize the great potential of MHC tetramers for selective immunotherapy in allograft rejections [42].

Peptide-loaded MHC molecules have a high affinity to bind the corresponding TCR on antigen-specific T cells. Some novel approaches took advantage of this ability to deplete T cells in an antigen-specific manner. Actinium 225-conjugated MHC tetramers have been demonstrated to bind to corresponding CD8⁺ T-cell lines and thereby inducing apoptosis in T cells via α -particle emission. By contrast, T cells specific for irrelevant antigens were not depleted [43]. Co-culture of T cells with ribosome inhibitor saporin-conjugated MHC tetramers resulted in the elimination of 75% of antigen-specific T cells after 72 h, whereas background apoptosis was observed in control co-cultures. Apoptosis induction was dependent on the saporin–MHC tetramer to T-cell ratio and the antigen-specific T-cell affinity [44]. With regards to these observations, there is evidence to suggest that MHC tetramers could be deployed for the generation of immune modulatory ‘guided missiles’.

To overcome problems related to autologous cell-based treatment strategies, many investigators promoted the development of artificial APC (aAPCs). Currently, aAPCs are mainly used for the expansion of antigen-specific CD4⁺ and CD8⁺ T cells for adoptive immunotherapy of chronic infections and cancer. Such studies aim to infuse *ex vivo* activated and expanded autologous antigen-specific or tumor-reactive T cells into donors receiving standard medical treatment [45–48].

Studies focusing on the development of an optimal aAPC phenotype have utilized artificially engineered cells [49,50] or other scaffolds such as liposomes [40,51] and latex or magnetic beads [52–54]. First, nonspecific activation and expansion of T cells using anti-CD3 and anti-CD28 mAbs or 4-1BBL-coated aAPCs [49,55] have been rapidly replaced by aAPCs utilizing soluble HLA molecules to improve T-cell–APC interaction [40,52–54,56]. In addition, costimulatory signals were also directly provided by such aAPCs through immobilization of antibodies including anti-LFA-1, anti-CD80 or anti-CD86. Moreover, recombinant production of different HLA class I and II molecules rapidly extended the capacity for the creation of a customized antigen-specific aAPC. Immobilization of HLA molecules onto beads or liposomes enhanced the expansion of antigen-specific T cells in mouse and human *in vitro* systems. Thus, different combinations of costimulatory signals and different amounts of HLA molecules were tested (reviewed in [57]).

One such aAPC was paving the way for the development of the KaAPC. Using an aAPC generated by immobilization of HLA-A2–Ig dimer molecules and anti-CD28 mAb on to paramagnetic epoxy beads, human antigen-specific CD8⁺ T cells have been successfully expanded *in vitro*. Antigen-specificity was ensured through loading of HLA-A2–Ig dimer molecules with peptides of interest. Expansion and specificity of antigen-specific CD8⁺ T cells was comparable or even improved compared with expansion protocols relying on classical peptide-pulsed DCs. Furthermore, peptide-pulsed target cells were efficiently lysed by aAPC-expanded CD8⁺ T cells [54]. These aAPCs generated with an B7.1-Ig instead of an anti-CD28 mAb augmented the activity of adoptively transferred cytotoxic T lymphocytes and

significantly delayed tumor growth in an *in vivo* treatment model of subcutaneous melanoma [58]. Therefore, this aAPC technology has been proved functional *in vitro* and *in vivo*, demonstrating that the combination of the MHC tetramer technology with a bead-based aAPC system could provide the advantage of specific T-cell recognition without the requirement of cell–cell interference.

The KaAPC platform technology

Based on the previous work on aAPCs [54], the KaAPC concept is founded on three pre-requisites:

- The backbone of the KaAPC consists of a paramagnetic epoxy bead, which is inexpensive, labor efficient and easy to handle;
- Antigen specificity of the KaAPC is warranted by a covalently linked HLA-A2–Ig dimer molecule, which can be loaded with different HLA-A2 restricted peptides;
- Apoptosis induction is mediated via an anti-Fas mAb utilizing the Fas/FasL signaling pathway in activated antigen-specific T cells.

Based on these requirements, a KaAPC phenotype displaying maximal antigen-specific depletion of CD8⁺ T cells and minimal T-cell activation at the same time has been considered as the ideal KaAPC phenotype (Figure 1D).

The phenotypical and functional characterization of this optimal KaAPC revealed an antigen-specific depletion of T cells by corresponding peptide-loaded KaAPCs. KaAPCs unloaded or loaded with an irrelevant peptide did not deplete antigen-specific or polyclonal activated Fas⁺ CD8⁺ T cells. Furthermore, stimulation of antigen-specific T cells with unloaded or peptide-loaded KaAPCs did not result in T-cell activation. Neither the expression of CD107a in co-cultured T cells nor a significant production of proinflammatory cytokines could be detected. Further experiments revealed that the amount of T-cell apoptosis was directly related to the amount of KaAPCs used in T-cell co-cultures. In addition, time course experiments indicated that 30 min of co-culture was sufficient to induce almost maximal apoptosis in antigen-specific Fas⁺ CD8⁺ T cells. Using a novel four-color flow cytometric assay [59], it could be demonstrated that KaAPCs selectively deplete corresponding antigen-specific T cells, even within a mixture of T cells with heterogeneous antigen specificities. At the same time, no relevant induction of apoptosis could be detected in T cells with nonrelevant antigen specificities.

Taken together, the collective data on KaAPCs demonstrated that apoptosis induction in antigen-specific T cells was dependent on the delivery of a combined signal via the TCR and Fas. Both signals could only be sufficient provided that KaAPCs also presented the corresponding peptide, whereas KaAPCs loaded with an irrelevant peptide failed to mediate these two signals (Figure 2) [60].

Therefore, KaAPCs in their present form prove efficient artificial *in vitro* guided missiles for the modulation of human antigen-specific T-cell responses.

KaAPC: a possible future?

Killer artificial antigen-presenting cells, conceived and developed as antigen-specific bead-based killer APCs, combine the advantages of the bead-based aAPC technology with the Fas/FasL signaling pathway to eliminate T cells in an antigen-specific fashion without hampering the T-cell repertoire.

Compared with cell-based approaches utilizing autologous APCs, generation of KaAPCs is much easier and less time consuming, preserving labor and resources. KaAPC phenotypes were stable over a long period, and the ‘off the shelf’ nature could make them an instant treatment possibility. Furthermore, signal strength and composition of the KaAPCs can be better controlled than on APCs, allowing a higher reproducibility. In addition, KaAPCs do not underlie gene-regulation issues. Furthermore, the quality of KaAPCs is not dependent on patient conditions as is required for the cell-based expansion protocols. Furthermore, KaAPCs cannot be eliminated through para- or auto-crine activation of the Fas/FasL signaling pathway. In addition, the functional capacity and the number of KaAPCs are not compromised by effector functions of cytotoxic T lymphocyte.

Killer artificial antigen-presenting cells can be customized through loading of defined peptides, avoiding the danger of tolerance induction or secondary immune responses to antigens other than the loaded peptides. However, this also represents one of the major limitations of this technology. Since KaAPCs do not phagocytose, process and present antigen, it will be necessary that at least the immune-dominant disease-relevant antigens are identified. Autologous cell-based strategies will not have such limitations as they present the complete repertoire of naturally occurring antigens. To date, many HLA-A2-restricted antigens have already been identified that account for several autoimmune diseases (Table 1). The fact that 40–50% of Caucasians feature HLA-A2 molecules in their individual HLA phenotype promises the treatment of up to 50% of all Caucasians, and the combination of 5–7 different HLA class I molecules would already cover more than 90% of the whole population. Therefore, therapeutic efficiency could be improved by the loading of different disease-related HLA-A2-restricted antigens onto the KaAPC. The almost exclusive usage of HLA-A2 molecules so far is not a limitation of this strategy alone, but cannot hide the fact that epitope mapping and peptide identification would have to be performed for other HLA class I and II subclasses to ensure an effective antigen repertoire for the generation of functional KaAPCs. It might be beneficial that at the onset of an autoimmune disease, only a few T-cell clones are activated. Therefore, a potential KaAPC treatment should be initiated in the early phase of an autoimmune disease if possible, preventing the antigen spreading observed at later disease stages [61–63].

However, before considering a clinical application of KaAPCs, several obstacles have to be acknowledged. Does the latex-coated ferrous paramagnetic core of KaAPCs have any side effects when applied to *in vivo* systems? These utilized beads are indeed available in a ‘good manufacturing practice’ grade but potential adverse effects due to size, coating and distribution have still to be evaluated. Initial aAPC data that proved *in vivo* functionality and biodistribution [58] encourage a potential *in vivo* application of KaAPCs. However, it is not yet clear if aAPC were actively directed towards tumor sites and tumor antigen-specific T cells or if the site of injection mainly determined the pattern of aAPC distribution. Hence, to date, it is nearly impossible to define the exact amount or the application strategy of KaAPCs for a potential future *in vivo* treatment. In addition, one might envisage the application of KaAPCs for purging of antigen-specific T cells *ex vivo*. In light of these limitations, future progress should focus on the development of biodegradable KaAPCs more suitable for potential *in vivo* studies. To this end, particles should be considered that have already been applied in pharmacological *in vivo* studies for targeted drug delivery [64] including liposomes. Promising results have been achieved utilizing liposomes for aAPC generation [40,51,65,66], supporting a possible transfer to the KaAPC technology.

Furthermore, the current KaAPC phenotype depleted highly activated antigen-specific T cells only. However, it remains to be elucidated if the present KaAPC phenotype might deplete T cells of different activation statuses. Since KaAPCs do not display costimulatory molecules and we did not observe any T-cell activation in co-cultures, it appears unlikely that this

phenotype will be able to deplete naive antigen-specific T cells. By contrast, killer APCs display the whole range of costimulatory molecules leading eventually to T-cell activation.

In addition, the anti-Fas mAb immobilized on to KaAPCs could interfere with other Fas⁺ cells, causing unspecific depletion of cells other than T cells, including hepatocytes. Therefore, modification of the apoptosis-inducing signal loaded onto KaAPCs should be considered. For this purpose, ligands or mAbs targeting other receptors of the TNF-receptor superfamily appear to be suitable apoptosis [67,68] or tolerance inducers [69]. Supporting this concept, Hirata *et al.* demonstrated that genetically modified DCs presenting myelin oligodendrocyte glycoprotein (MOG) peptide in the context of MHC class II molecules in the presence of TNF-related apoptosis-inducing ligand or programmed death-1 ligand expression significantly reduced T-cell responses towards MOG, resulting in reduced cell infiltration into the spinal cord and reduced severity of MOG peptide-induced experimental autoimmune encephalomyelitis [70].

Furthermore, the addition of costimulatory molecules or the combined use of HLA class I and II signals is conceivable and potentially of benefit for a possible future therapeutic application [71]. Moreover, by introducing other molecules such as CD137, the KaAPC technology could be deployed for the depletion of alloreactive T cells, since these cells could already be characterized *ex vivo*, generated and depleted [72–75]. A combination of both HLA class I and II molecules eventually promote the complete depletion of alloreactive T cells, which would facilitate the treatment of graft rejection and might reduce its incidence. However, these concepts are still ‘dreams of the future’.

Future perspective

Killer APCs, guided missiles and inhibitory tetramers together with our KaAPCs hold promise for an effective antigen-specific treatment of T-cell-related autoimmune diseases. But clinical application in the future will depend on several factors. First, disease-related antigens and T-cell populations have to be identified and characterized to allow a targeting as specific as possible. Second, the issue of which strategy will be most efficient *in vivo* with minimal potential side effects is still yet to be evaluated. To date, the KaAPC technology is one of the first strategies displaying antigen-specific deletion of human T cells *in vitro*, but other strategies, such as inhibitory tetramers [43] or nonvirally transduced cell-based settings [76], could be better alternatives in terms of biocompatibility.

Therefore, after successful proof-of-concept, the future of antigen-specific treatment strategies fundamentally relies on an improved biocompatibility and specificity at the same time. However, given the rapid development of biomaterials, there is a fair chance that antigen-specific depletion strategies will enter the clinic within the next few decades.

Executive summary

Current autoimmune & chronic infection treatments

- Most current treatment strategies of autoimmune diseases rely either on corticosteroids or immunosuppressive drugs, such as purine analogs, alkylating agents and calcineurin inhibitors, which do not result in cure of the primary immunological malfunction.
- New biological agents have been introduced, such as monoclonal antibodies (mAbs), targeting inflammatory effector molecules, defined proteins or receptors expressed on the cell surface.

- Other mAbs targeting cytokines, receptors, integrins and chemokines have been approved or are currently under investigation in clinical trials.
- However, these strategies are not restricted to pathogenetically relevant cells or mechanisms, leading to potentially severe side effects.

Cell-based depletion strategies utilizing the Fas/Fas ligand signaling pathway

- Many *in vitro* and *in vivo* animal model utilized B-cell lines, macrophages and dendritic cells (DCs) as killer antigen-presenting cells (APCs) to demonstrate antigen-specific depletion of T cells.
- Killer APCs were successfully tested in *in vivo* animal models resembling features of rheumatoid arthritis, allogeneic transplantation and allergy, as well as in models for studying antigen-specific T-cell responses.
- It has been demonstrated that human killer APCs do not deplete naive human T cells but eliminate alloantigen-specific T cells, thereby preserving protective antiviral T-cell responses.

Artificial concepts for antigen-specific immunotherapy

- Development of cell- and bead-based artificial APCs:

Unspecific (anti-CD3 and anti-CD28) and specific approaches utilizing HLA class I and II molecules in combination with different costimulatory molecules (anti-CD80, anti-CD86 and anti-LFA-1) have been tested.

Artificial APCs (HLA-A2-Ig; B7.1-Ig) display a prominent *in vivo* functionality by augmentation of adoptively transferred cytotoxic T lymphocyte and consequently delayed tumor growth.

- Depletion strategies utilizing MHC-tetramers linked to cell-toxic agents:

Actinium 225-conjugated MHC tetramers specific for human viral antigens have been demonstrated to deplete antigen-specific T cells via α particle emission.

Ribosome inhibitor saporin-conjugated MHC tetramers resulted in the depletion of antigen-specific murine T cells.

Killer artificial antigen-presenting cell technology

- Killer artificial antigen-presenting cells (KaAPC)s are generated by covalently bound HLA-A2-Ig and anti-CD28 mAbs onto the surface of paramagnetic epoxy beads.
- KaAPCs deplete antigen-specific T cells in an antigen-specific manner, even from T-cell mixtures of heterogeneous antigen specificities.
- Depletion efficiency is dependent on the amount of KaAPC used and the co-culture interval, and is not related to activation-induced cell death.

Advantages over cell-based approaches

- No induction of tolerance to unwanted antigens.
- No negative impact on the protective T-cell immune response.
- Not susceptible to cytotoxic effector functions of cytotoxic T lymphocyte.
- ‘Off the shelf’ nature.
- Time-, cost- and labor-saving technology.

Shortcomings of this new concept

- Paramagnetic backbone is approved for *in vivo* studies but data related to biodistribution, clearance and toxicity are sparsely published.
- Efficient use of KaAPCs requires the identification of immune-dominant disease-related antigens.
- The current KaAPC phenotype efficiently depletes only highly activated T cells.

Conclusion

- Proof-of-concept of KaAPC-mediated human antigen-specific T-cell elimination utilizing a HLA-A2–Ig dimer and anti-Fas mAb has been accomplished.
- KaAPC technology could provide a robust and highly flexible platform for individual treatment strategies if the utilization of other costimulatory or apoptosis-inducing molecules is applicable.
- For a putative clinical application, the KaAPC technology has to first be adapted to a biodegradable or biocompatible backbone and also requires better *in vivo* characterization in terms of distribution, toxicity and clearance.

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Figure 1. Possible T-cell-antigen-presenting cell interactions

(A) If solely a TCR–HLA interaction occurs without further stimulation, functional anergy is induced in interacting T cells. (B) The additional presentation of a prominent costimulatory signal leads to efficient activation and induction of clonal expansion in T cells. (C) Together with these two signals, killer APCs provide an additional ‘third signal’ as these cells express FasL that induces apoptosis in Fas⁺ T cells. Killer artificial APCs are the synthetic embodiment of a cell-based killer APC. (D) These constructs simultaneously provide an antigen-specific signal (HLA-A2–Ig dimer) and an apoptosis signal (anti-Fas mAb) resulting in efficient antigen-specific depletion of Fas⁺ T cells.

APC: Antigen-presenting cell; FasL: Fas ligand; Ig: Immunoglobulin; mAb: Monoclonal antibody; TCR: T-cell receptor.

Figure 2. Analysis of antigen-specific elimination of human cytotoxic T lymphocytes with different antigen specificities

Autologous PKH26-stained FluM1-specific CTL and PKH67-stained CMV_{pp65}-specific CTLs were mixed at a 1:1 ratio and then co-cultured with unloaded (unloaded KaAPC), Mart-1-loaded (Mart-1 KaAPC) or CMV_{pp65}-loaded (CMV_{pp65} KaAPC) KaAPCs at a 1:1 ratio. After 48 h, co-cultures were harvested and additionally stained with annexin V and 7-actinomycin D. Anti-Fas mAb-stimulated mixed T-cell cultures served as positive control. Apoptosis was determined by differential gating on PKH26-stained and PKH67-stained T-cell populations, respectively (view protocol [60]). Numbers indicate the percentages of gated cells.

CMV: Cytomegalovirus; CTL: Cytotoxic T lymphocyte; KaAPC: Killer artificial antigen-presenting cell; mAb: Monoclonal antibody.

Table 1

Exemplarily overview of already known human HLA-A*0201 restricted disease-related antigens.

Disease	Antigen	Position	Sequence	Ref.
Type 1 diabetes	GAD65	114–123	VMNILLQYVV	[77]
		172–180	SLSPLQAEI	[78]
		482–490	SLAAGVKLL	[78]
		797–805	MVWESGCTV	[79]
	GFAP	143–151	NLAQDLATV	[80]
		192–200	SLEEEIRFL	[80]
		214–222	QLARQQVHV	[80]
	IAPP	5–13	KLQVFLIVL	[78,80,81]
		9–17	FLIVLSVAL	[78,80]
	IGRP	152–160	FLWSVFWLI	[78,80]
		211–219	NLFLFLFAV	[82]
		215–223	FLFAVGfYL	[78,80]
		222–230	YLLLRVLNI	[82]
		228–236	LNIDLLWSV	[83]
		265–273	VLFGLGFAI	[83]
		293–301	RLLCALTSI	[80]
	Insulin	B 9–18	SHLVEALYLV	[84]
		B 10–18	HLVEALYLV	[78,84–87]
		B 18–27	VCGERGFFYT	[84,85]
		C 20–28	SLQPLALEG	[84,87]
		C 25–33	ALEGLQKR	[84]
		C 29 – A 5	SLQKRGIVEQ	[84,87]
		A 1–10	GIVEQCCTSI	[84,87]
		A 12–20	SLYQLENYC	[84,87]
		L 2–10	ALWMRLPL	[78]
Multiple sclerosis	MBP	87–95	VVHFFKNIV	[88,89]
		110–118	SLSRFSWGA	[88–90]
	MAG	287–295	SLLLELLEEV	[90]
		509–517	LMWAKIGPV	[90]
		556–564	VLFSDFRI	[90]
	MOG	43–51	ALVGDEVEL	[89]
		97–105	RTELLKDAI	[89]
		104–112	AIGEGKVTI	[89]
		143–151	KVEDPFYWV	[89]
		157–166	LLAVLPVLLI	[89]
		164–171	VLLQITV	[89]
		185–193	KLRAEIENI	[89]
		203–211	RVPCWKITL	[89]
		248–255	SLCYKQRI	[89]

Disease	Antigen	Position	Sequence	Ref.
	PLP	263–271	EATRGRGGL	[89]
		3–11	LLECCARCL	[89]
		63–72	NVIHAFQYVI	[89]
		80–88	FLYGALLLA	[90–92]
	TAL	223–231	NLLSICKTA	[89]
		168–176	LLFSFAQAV	[93]
Primary biliary cirrhosis	PDC-E2	159–167	KLSEGDLLA	[94]
		165–174	LLAEIETDKA	[95]