

Artificial antigen-presenting cells: artificial solutions for real diseases

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Adoptive immunotherapy, which involves the transfer of autologous antigen-specific T cells generated *ex vivo*, is a promising strategy to treat a variety of life-threatening diseases. Unfortunately, current approaches for generating sufficient numbers of antigen-specific T cells lack the ability to serve as reproducible and economically viable methods. This has spurred the development of both cell- and non-cell-based artificial antigen-presenting cells to alleviate problems associated with peptide-loaded dendritic cells in current approaches to adoptive immunotherapy. Here, we review new strategies for the *ex vivo* generation of antigen-specific T cells and their clinical application. These new approaches have the potential to spearhead a new era of successful adoptive immunotherapy for cancer and infectious diseases.

Introduction

A major goal in immunotherapy is to generate an effective cell-mediated immune response. Efforts to stimulate therapeutic immune responses against cancer and infectious diseases can be broadly categorized into active or adoptive immunotherapy. Active immunotherapy represents a diversity of approaches aimed at introducing antigens into patients in an immunogenic form, either to break tolerance or to activate the 'cryptic' T-cell repertoire [1]. Adoptive immunotherapy involves the *ex vivo* stimulation and expansion of antigen-specific T cells and re-transfer of the expanded T cells into patients [2]. This has been mostly accomplished using autologous antigen-presenting cells (APCs), which are highly variable in quality and quantity, and are often impaired secondary to the primary disease. In addition, generation of APCs such as dendritic cells (DCs) is labor intensive and expensive. Therefore, many investigators have started to develop artificial (a)APCs. Advances in the development of aAPCs have been reviewed earlier [3,4], and the different technological advances in adoptive immunotherapy, including aAPCs and T-cell receptor (TCR) gene transfer, have been summarized [4]. Here, we discuss the molecular basis for T-cell activation and how this process affects aAPC design and their potential for usage in clinical applications.

The world of T-cell activation: requirements for successful T-cell stimulation

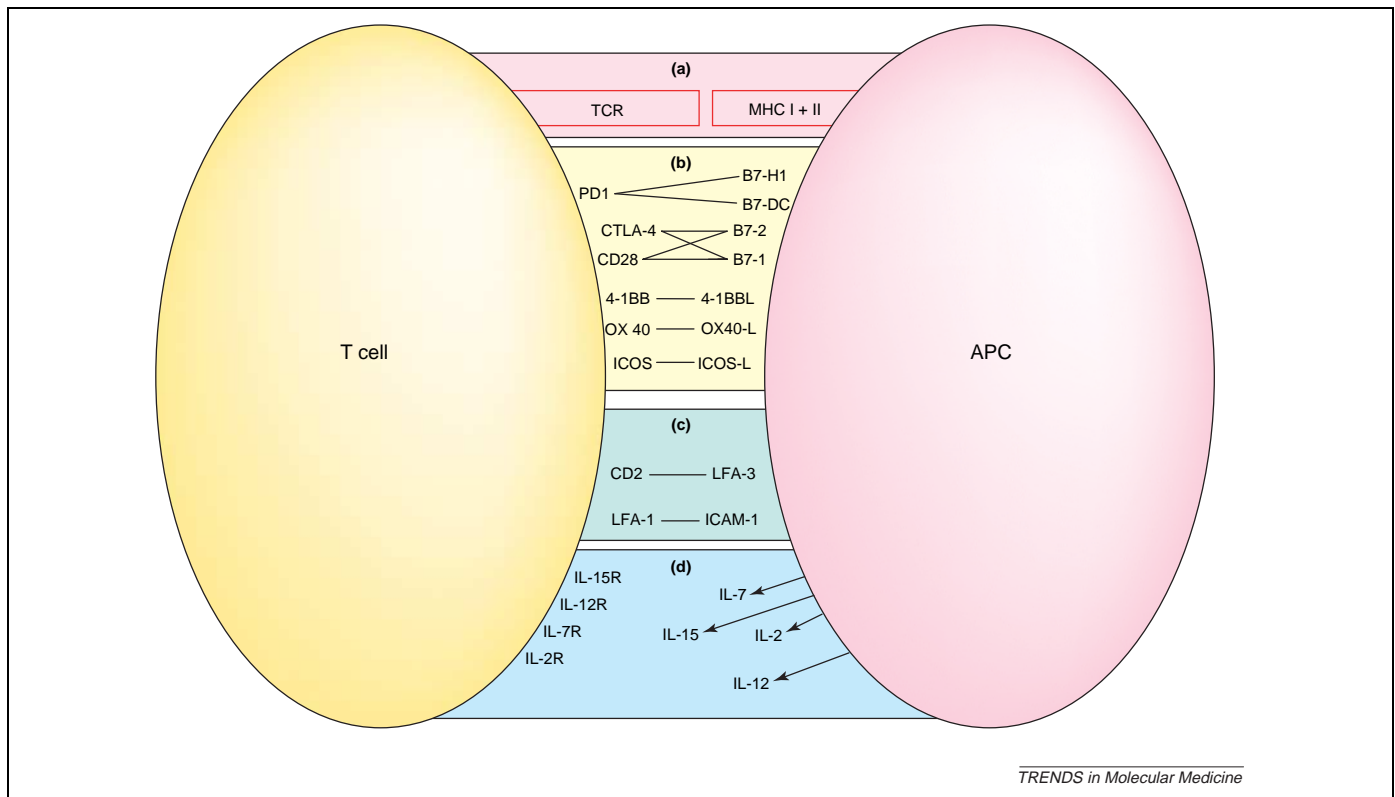
Successful adoptive immunotherapy relies on optimal T-cell stimulation, which requires engagement of the TCR through the major histocompatibility complex (MHC) bound to peptide (Figure 1a), together with at least one interaction of a costimulatory molecule with an appropriate ligand on the T cell (Figure 1b). The most potent and best-studied costimulatory molecules are B7-1 and B7-2, which bind to CD28 [5,6] on the T cell and induce proliferation. Conversely, B7 molecules might also bind cytotoxic T lymphocyte antigen 4 (CTLA-4) molecules on activated T cells and induce apoptosis in those T cells. Similar reports have been published for the molecules B7-H1 and B7-DC, which induce proliferation through the interaction with an as-yet-unknown receptor termed PD-2 on T cells [7,8], whereas binding to PD-1 induces negative regulation of cytokine production and proliferation [9,10]. This mechanism enables the immune system both to proliferate at the beginning of an immune response and to downregulate T-cell proliferation as the infection is cleared. Additional interactions might also modulate T-cell activation, including T-cell subtype differentiation, induction of maximal proliferation and prevention of apoptosis. Potential molecules for these interactions are other costimulatory molecules such as ICOS, 4-1BBL and OX40 [6,11–15].

Another important group of molecules are the adhesion molecules, which include leukocyte function-associated antigen (LFA) and intercellular adhesion molecule (ICAM) [16,17]. These prolong the interaction time between the T cell and APC (Figure 1c). In addition to these direct interactions, there are also indirect interactions through soluble factors, cytokines and chemokines, such as interleukin 2 (IL-2) and IL-15 (Figure 1d). These indirect interactions might also influence T-cell activation and inhibition. Soluble factors function not only in T-cell differentiation and activation but also in trafficking to the site of infection or to the tumor.

As described above, over the past decade many new costimulatory molecules and factors have been identified, offering new insights into T-cell activation and regulation. This knowledge has facilitated the rationale for the development of aAPCs to study and manipulate T-cell activation. For example, the introduction of 4-1BBL [12] [a member of the tumor necrosis factor (TNF) family] on a

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Figure 1. Overview of potential T cell–APC interactions. **(a)** T-cell stimulation requires engagement of the TCR through the major histocompatibility complex (MHC) bound to peptide, together with **(b)** at least one interaction of a costimulatory molecule with an appropriate ligand on the T cell. The most potent costimulatory molecules are B7-1 (CD80) and B7-2 (CD86) on APCs, which interact with CD28 and CTLA-4 on T cells to mediate positive or negative regulation. **(c)** Another important group of molecules are the adhesion molecules, which include LFA and ICAM. These prolong the interaction time between the T cell and APC. **(d)** In addition to these direct interactions, there are also indirect interactions through soluble factors, cytokines and chemokines, such as interleukin 2 (IL-2) and IL-15. These indirect interactions might also influence T-cell activation and inhibition.

nonspecific aAPC together with anti-CD28 and anti-CD3 enabled researchers to expand CD8⁺ and CD4⁺ T cells; by contrast, without 4-1BBL, only CD4⁺ cells could be expanded [18]. One could also envision a negative regulatory aAPC for the treatment of autoimmune disease using inhibitory molecules such as B7-H4 [19,20] or Fas ligand (FasL) [21,22]. Thus, recent scientific developments have driven novel aAPC formulations, and it might even be possible to develop aAPCs that are specifically useful for induction of antigen-specific T cells or T-cell expansion or apoptosis inhibition. A third generation might be able to control unwanted autoreactive T-cell proliferation. Although autologous APCs (DCs) combine all of these features if controlled through the immune system, current knowledge and technology does not enable us to control and regulate them *ex vivo*. Therefore, aAPCs constitute a valuable tool whereby current knowledge can be used to optimize formulation.

Developments in adoptive immunotherapy

Adoptive immunotherapy might enable a more directed approach for the supportive treatment of a variety of cancers and infectious diseases, especially in the setting of both organ and bone marrow transplantation. Adoptive immunotherapy requires rapid and reproducible methods for induction, and expansion and enrichment of specific cytotoxic T lymphocyte (CTL) lines or clones from low precursor frequencies to clinically relevant numbers. Traditionally, this has been expensive and labor intensive,

thus prompting the development of a variety of novel techniques for adoptive immunotherapy.

Adoptive transfer of antigen-specific T cells generated *ex vivo* was first described using cytomegalovirus (CMV)-specific CTLs expanded *ex vivo* for treatment of CMV infection in immunocompromised allogeneic bone marrow transplant recipients [23,24]. These investigators were able to show that protective T-cell immunity against CMV had been restored [23,24]; none of the immunocompromised patients developed CMV viremia or CMV disease. On the basis of success with CMV, adoptive T-cell transfer immunotherapy studies for Epstein–Barr virus (EBV)- and human immunodeficiency virus (HIV)-related disease [25–30] have been performed with encouraging results.

Adoptive immunotherapy has also been tried in the treatment of tumors. Whereas initial studies used polyclonal nonspecific T cells, later studies have used antigen-specific T cells. In 1988, Rosenberg expanded autologous tumor-infiltrating lymphocytes (TILs) in nonspecific fashion, *ex vivo*, and re-infused them into melanoma patients [31]. These re-infused TILs were able to home to the tumor sites *in vivo*. In some cases, the transferred TILs seemed to induce tumor shrinkage directly and some of the recipients developed evidence of T-cell-mediated vitiligo. More recently, adoptive transfer of melanoma-specific TILs expanded *ex vivo* after non-myeloablative pretreatment of melanoma patients resulted in massive tumor shrinkage [32]. One patient achieved complete remission. In these patients, lysis was also detected of

Table 1. APC versus aAPC: what makes the difference?

APC/aAPC	Antigen specific	Cell based	Antigen uptake	Antigen processing	Number of different MHC class I/II	Secrete cytokines	Costimulation	Storage	Availability	Refs
Autologous DC	Yes	Yes	Yes	Yes	6/6	Yes	All kinds of costimulation if fully mature and activated	Difficult long-term storage; is likely to be challenging	Always limited and, in case of disease, often impaired	[34,41–44]
aAPC: CD3/CD28-based bead (see Figure 2a in main text)	No	No	No	No	0/0	No	Anti-CD28	Can be stored in large numbers at 4°C	No limitation	[54]
aAPC: K32 (see Figure 2b in main text)	No	Yes	No	No	0/0	No	4-1BBL, anti-CD28	Liquid nitrogen for long-term storage	No limitation; cells need to be cultured and antibody loaded	[18]
aAPC: fibroblast/ <i>Drosophila</i> -based cell line (see Figure 2c in main text)	Yes	Yes	No	Yes	1/0	No	B7-1, CD54, CD58	Liquid nitrogen for long-term storage	No limitation; cells need to be cultured	[58–60]
aAPC: latex-bead based (see Figure 2d in main text)	Yes	No	No	No	1/0	No	B7-1, B7-2	Can be stored in large numbers at 4°C	No limitation	[62,65]
aAPC: HLA-Ig based (see Figure 2e in main text)	Yes	No	No	No	1/0	No	Anti-CD28	Can be stored in large numbers at 4°C	No limitation	[53,66]
aAPC: liposome based (see Figure 2f in main text)	Yes	No	No	No	0/1	No	No costimulation	N/A	N/A	[64]
aAPC: MHC class II tetramer-based bead (see Figure 2g in main text)	Yes	No	No	No	0/1	No	Anti-CD28	Can be stored in large numbers at 4°C	No limitation	[52,72]

normal tissue expressing Mart-1 (for ‘melanoma antigen recognized by T cells 1’) [33] in an autoimmune fashion, in addition to vitiligo. In another study, the effectiveness of melanoma-specific CTLs induced and expanded *ex vivo* was demonstrated as survival of Mart-1-specific T cells *in vivo* for several weeks. In addition, the infused Mart-1-specific CTLs could be detected preferentially at the site of the tumor [34]. Studies in other malignancies are in progress and also show promise [35–40].

Current issues: the argument for artificial solutions

Currently, the most potent and commonly used professional APCs are autologous DCs [34,41–44]. However, there are a variety of limitations related to the use of autologous DCs. Their accessibility is limited and often requires several rounds of leukapheresis, which is burdensome on patients, to obtain sufficient numbers of precursor cells from the patient for *in vitro* generation of DCs. The process of *in vitro* generation of DCs is expensive and both time and labor intensive. In addition, the quality of the DCs generated *in vitro* is highly variable as a result of the pretreatment and the disease itself. For example, it has been shown in patients with breast cancer and multiple myeloma that, secondary to the disease, the number and function of DC precursors and the resulting DCs is diminished [45–48].

To avoid using autologous stimulator cells for antigen-specific T-cell expansion, one approach has been to use T-cell clones, which can be expanded to clinically relevant numbers using nonspecific anti-CD3-mediated stimulation. However, expanded T-cell clones have often reached the Hayflick limit [49,50], resulting in reduced functional capacity after re-infusion into the patient. As a

result of these limitations associated with the use of autologous APCs, new DC-independent methodologies for an ‘optimal T-cell activation’ are being developed (see Table 1 for a comparison of aAPCs with DCs). This article summarizes current developments in adoptive immunotherapy, focusing on the development of aAPCs.

aAPCs for adoptive immunotherapy

Two recent approaches have had a dramatic impact on adoptive immunotherapy. One approach has focused on the nonspecific expansion of antigen-specific T-cell clones, isolated from either TILs or DC-induced antigen-specific T-cell lines. The other approach is the development of a variety of alternative antigen-specific aAPCs to enhance the generation of antigen-specific T cells from precursor T cells. Although each approach has limitations, together they highlight certain pivotal requirements (Box 1) that will markedly enhance the clinical use of aAPCs in adoptive immunotherapy.

Nonspecific aAPCs

Cell- and bead-based aAPCs have been developed for the nonspecific expansion of T cells [18,51,52]. These nonspecific aAPCs have been used to expand antigen-specific T-cell clones and lines. Initially, the bead-based aAPCs were made using magnetic Dynal beads (<http://www.dynal.no>), which were coated with anti-CD3 and anti-CD28 monoclonal antibodies (mAbs) (Figure 2a). More recently, Miltenyi (<http://www.miltenyibiotec.com>) developed a similar system for nonspecific T-cell expansion, based on anti-CD3/anti-CD28 and anti-CD28 coupled to magnetic beads.

These nonspecific aAPCs have a differing capacity to expand CD4⁺ T cells versus CD8⁺ T cells. Thus, this

Box 1. Pivotal requirements for an optimal clinical-grade aAPC

An aAPC should meet the following criteria:

- (i) simple and standardized manufacture with reproducible results;
- (ii) long shelf half-life, enabling economical manufacture of large amounts of GMP-grade beads;
- (iii) stimulation should not be dependent on the state of the patient's DCs;
- (iv) 'one-type-fits-all'; i.e. the same aAPC can be loaded with different peptides and therefore can be used to treat a variety of diseases;
- (v) easy to exchange or add in costimulatory molecules to optimize T-cell effector function as they are identified (see Figure 1 in main text).

approach was only effective for expansion of CD4⁺ T cells and not for long-term, >10 days, expansion of CD8⁺ CTLs. In addition, even during short-term 1–2-week expansion, the expanded CD8⁺ CTLs lost their antigenic specificity [53]. The mechanism for the loss of antigen specificity is unknown. It might be that contaminating polyclonal naive T cells have a higher affinity for the nonspecific stimulus T cells or they simply have a higher proliferative capacity than the antigen-specific memory cells that have already undergone multiple rounds of replication. Even the addition of T-cell growth factors such as IL-2 to the culture medium did not overcome these limitations. These findings indicate that the nonspecific bead-based approach can be used most effectively for the polyclonal expansion of CD4⁺ T helper (Th) cells only, and is currently being evaluated in a clinical trial for HIV therapy [54]. However, these nonspecific bead-based aAPCs are not likely to be useful for the large-scale expansion to clinically relevant numbers of antigen-specific cytotoxic CD8⁺ T cells.

An alternate nonspecific cell-based aAPC (K32 cells) has also been developed using the MHC class I-negative leukemia cell line K562, transfected with the costimulatory molecule 4-1BBL and the low-affinity Fc gamma receptor CD32 [18] (Figure 2b). Stimulation of 4-1BB, which is expressed on activated T cells, through its natural ligand (4-1BBL) results in decreased apoptosis, increased production of IL-2 and therefore facilitates T-cell growth [55–57]. To stimulate T cells and to gain additional costimulatory capacity, anti-CD3 and anti-CD28 mAbs were bound to the surface of the K32 cells through CD32 molecules. Although this cell-based aAPC can be used for expansion of CD8⁺ T cells, there is still significant loss of antigenic specificity associated with this approach similar to that seen with anti-CD3/anti-CD28 beads [18], and underlines the importance of the pivotal requirements for an optimal aAPC (Box 1).

The frequency of transferred antigen-specific T cells is crucial for the efficacy of adoptive immunotherapy because transferred nonspecific T cells will presumably not help in the treatment of the primary disease and might even cause deleterious side effects, such as autoimmune diseases or graft-versus-host disease (GVHD). Using nonspecific aAPCs for expansion of antigen-specific T cells requires additional steps for the enrichment of the

antigen-specific CTL. Therefore, advances in aAPC formulation or culture conditions that streamline the generation of large amounts of highly antigen-specific CD8⁺ T cells will facilitate translation of adoptive immunotherapy into clinically relevant treatments of diseases.

MHC-class-I-based antigen-specific aAPCs

In addition to the nonspecific approaches to T-cell stimulation, several promising approaches for the specific induction and expansion of antigen-specific T cells are being studied. These advances can be split into MHC-class-I- and MHC-class-II-based aAPCs, and further subdivided into cell-based and 'off-the-shelf' acellular systems.

Early cell-based approaches developed aAPCs based on *Drosophila* cell lines (Figure 2c) [58], which were transduced with murine MHC class I molecules together with the costimulatory molecules B7-1 and ICAM-1. By coculturing unsorted normal spleen cells with the *Drosophila* cell-based aAPCs, stimulated T cells specifically killed tumor cells both *in vitro* and *in vivo*. However, insect cells are unstable at 37°C, resulting in massive self-destruction when cultured with T cells, causing a release of *Drosophila* antigens and a reduction in contact time. Nevertheless, the release of foreign antigens serves as a maturation stimulus for B cells, which then in turn can provide the necessary 'signal two' for T-cell activation [58].

Another cell-based approach is the use of the murine fibroblast cell line NIH/3T3, transduced with the costimulatory molecule B7-1, the adhesion molecules ICAM-1 and LFA-3, and a single HLA-peptide complex (HLA-A2) (Figure 2c) [59–61]. Using this system, investigators were able to show a 200–600-fold expansion of human CMV-specific T cells in about three weeks with 95% antigenic specificity. However, the T cells were sorted for specificity after the first round of stimulation to obtain the high purity needed for additional rounds of stimulation. This aAPC proved more valuable than autologous APCs in terms of antigenic specificity, total cell number, culture time and alloreactivity. In contrast to the system based on the *Drosophila* cell line, no additional feeder cells were used [60].

In acellular systems, aAPCs are usually based on latex microspheres [62,63] (Figure 2d), a magnetic bead [52,53] (Figure 2e,g) or liposomes [64] (Figure 2f). In one of the first bead-based systems, biotinylated murine MHC class I-peptide-single-chain constructs were coupled together with biotinylated costimulatory molecules B7-1 and B7-2 through streptavidin to the surface of latex microspheres [62,65] (Figure 2d). These engineered latex microspheres were able to stimulate antigen-specific T cells from transgenic mice. In addition, because this approach uses a single-chain MHC-peptide complex to ensure homogenous loading of the MHC molecules, each target peptide antigen would require a new transfection for expression of the desired single-chain MHC-peptide complex, thus limiting the generality of the approach.

Recently, a robust acellular aAPC was developed that can be used to induce and expand clinically relevant amounts of highly enriched antigen-specific T cells based on HLA-A2-Ig molecules and anti-CD28 antibodies

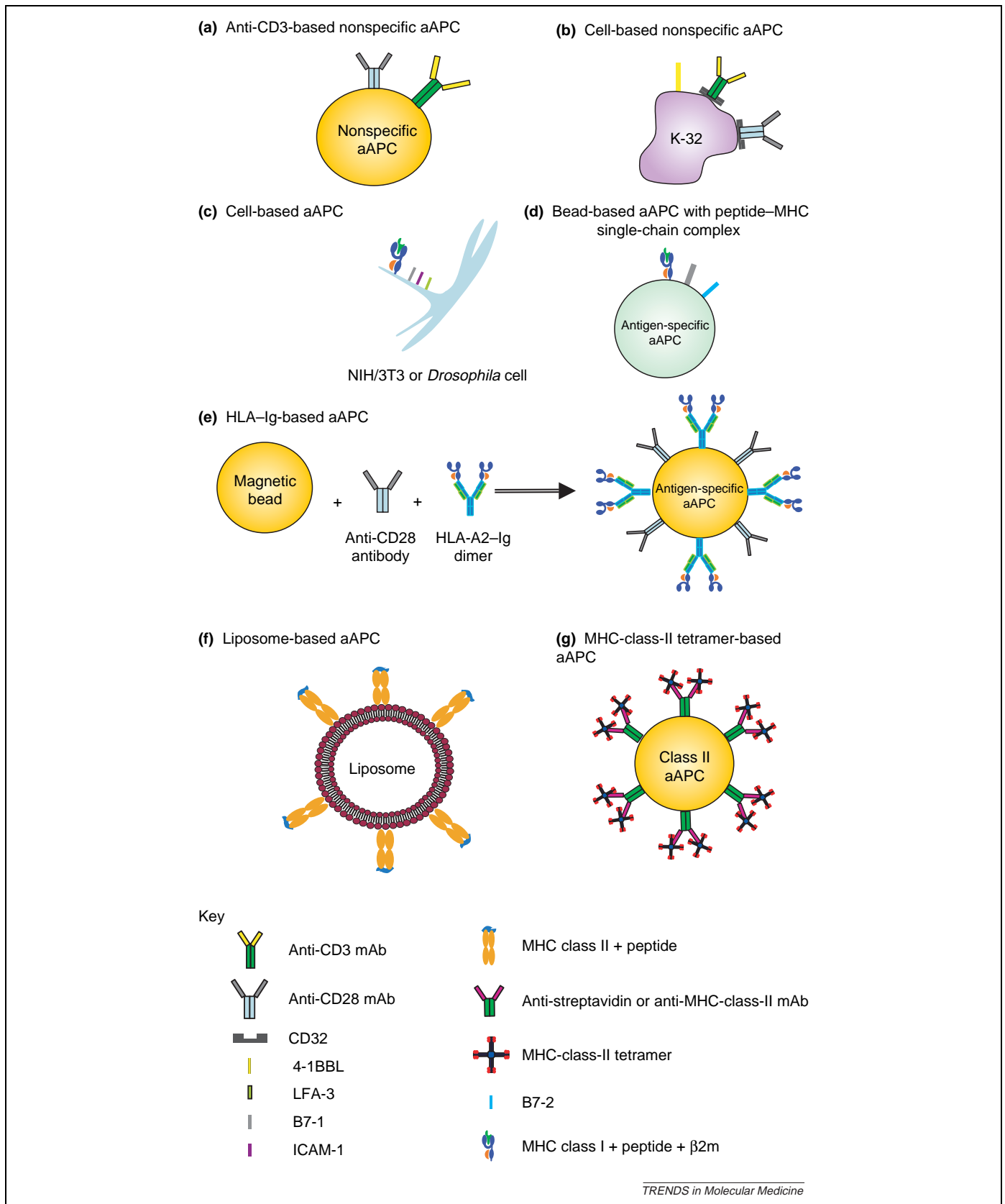


Figure 2. Schematic cartoons of different aAPC approaches as described in the text (a–g). Sizes and proportions are not to scale.

coupled to a magnetic bead [53,66]. In this system, once HLA-A2-Ig is coupled to the beads, one can exchange the peptide resident in the HLA-Ig molecule with any HLA-A2-restricted antigenic peptide. Thus, a single batch

of HLA-A2-Ig-based aAPCs can be loaded with a variety of different antigenic peptides for expansion of cells with different antigenic specificities, a pivotal requirement for optimal aAPCs (Box 1). Using this approach, it was

possible to generate antigen-specific T cells directed at clinically relevant tumor antigens such as the melanoma self-antigen Mart-1 or infectious disease antigens such as CMV_{pp65}. With this approach, an expansion of at least a million-fold Mart-1-specific CTLs was achieved in fewer than two months. Furthermore, HLA-Ig-based aAPCs induced CTLs directed at a subdominant peptide from the cancer testis antigen NY-ESO-1 and successfully recognized and lysed allogeneic tumor cells in an antigen-specific fashion [53]. Studies comparing the expansion of antigen-specific CTLs using either peptide-loaded HLA-A2-Ig-based aAPCs (Figure 2e) or anti-CD3/anti-CD28-based aAPCs (Figure 2a) showed a reduction of antigen-specific T-cell frequency when antigen-specific T cells were stimulated with anti-CD3/anti-CD28 beads [18], which was not seen using HLA-Ig-based aAPCs [53].

In summary, antigen-specific aAPCs have a clear advantage over nonspecific aAPCs. Because HLA-Ig-based aAPCs offer the option of loading with any peptide antigen restricted for specific HLA molecules, the same stock of 'off-the-shelf' aAPCs can be used for a variety of antigens and diseases. Thus, they have clear advantages over other approaches that require a new transfection or production of new single-chain constructs for each antigen.

MHC-class-II-based antigen-specific aAPCs

Approaches to generate antigen-specific Th cells are particularly interesting because it has been reported in several mouse models that Th cells are necessary to generate cytotoxic T memory cells [67,68]. Furthermore, it has been shown that tumor-specific Th cells support the CD8⁺ T-cell response, even when the tumor is MHC class II negative [69,70].

Initially, to make class II-specific aAPCs, investigators incorporated soluble mouse MHC class II molecules into liposomes [64]. These aAPCs were ~60–90 nm in diameter and stimulated ovalbumin (OVA)-specific T cells isolated from TCR-transgenic mice after loading the aAPC with the relevant peptide. Nevertheless, these aAPCs are too small to induce antigen-specific T cells efficiently when starting from naive cells. More recently, investigators have developed a similar system where they use MHC-peptide complexes in association with costimulatory molecules. This second generation of liposome-based aAPCs has been used to study the importance of clustered MHC microdomains, in addition to the positive effect of TCR pre-clustering on T-cell activation [71].

Recently, MHC-class-II-based aAPCs were developed using MHC monomers or tetramers bound either directly, through streptavidin, or through anti-MHC class II antibodies to a magnetic bead in combination with anti-CD28 [52,72] (Figure 2g). Such aAPCs could induce antigen-specific effector function in influenza-specific T cells. However, actual T-cell induction or expansion of antigen-specific T cells when starting from a bulk culture was not demonstrated. In addition, these studies showed that efficacy of the aAPC was only seen when the MHC was not coupled directly to the bead but rather indirectly through an antibody. This raises the possibility that spacing might be crucial for efficacy. In the HLA-A2-Ig-

based aAPC, the larger size of the complex [4] might fulfill the spacing requirements and facilitate its ability to stimulate T cells effectively.

Much progress has been made in the field of antigen-specific aAPCs for the generation of antigen-specific Th cells for adoptive immunotherapy. The most promising approaches are bead-based aAPCs. However, still more progress is needed to develop clinically relevant approaches for the generation of antigen-specific Th cells.

Clinical applications and patients safety: where we are and where we need to go

Several requirements are necessary to maximize therapeutic potential and minimize morbidity associated with adoptive T-cell transfer. T cells generated *in vitro* for adoptive transfer should be antigen specific, be able to home to the lymphoid tissue, and at the same time should not mediate GVHD. Furthermore, they must maintain the potential for *in vivo* expansion and be able to migrate into peripheral organs to mediate effector functions. Current studies using clinical-grade aAPCs are addressing these very questions.

Several groups have undertaken development of clinical-grade aAPCs for adoptive T-cell transfer. In initial studies for the treatment of patients with HIV, nonspecific anti-CD3/anti-CD28-based aAPCs were used to generate a large numbers of CD4⁺ T cells [54]. Currently, these anti-CD3/anti-CD28-based aAPCs are being used commercially by Xcyte (<http://www.xcyte.com>). Using this approach, it has been shown that, from peripheral blood mononuclear cells (PBMCs), isolated CD4⁺ T cells could be expanded on average ~37-fold. In addition, as a result of an unknown process connected to the anti-CD28 costimulation, the viral load decreased more than tenfold during cell expansion. In the first clinical studies, an average dose of 7×10^9 polyclonal CD4⁺ T cells, *in vitro* expanded with anti-CD3/anti-CD28 beads, were administered to HIV⁺ individuals in repeated infusions every 10 weeks for approximately two years. An increase in the CD4⁺ T-cell count, in addition to a decrease in CCR5⁺ cells, could be detected, indicating a reconstitution of the Th-cell pool and an increased resistance against HIV.

In another study, the anti-CD3/anti-CD28 beads were used for expansion of polyclonal CD4⁺ T cells in patients with non-Hodgkin's lymphoma [73]. The patients were pretreated with chemotherapy and hematopoietic cell transplantation (HCT) before they received a single infusion of nonspecifically expanded polyclonal CD3⁺ T cells. This study demonstrated complete remission in several patients. The response persisted for at least 33 months after completing the study. Three of the patients showed dose-dependent transient side-effects (congestive heart failure, diplopia, hemiparesis and fever) that were not in all cases strictly connected to the experimental protocol. These studies, although initial, give the first insights into the potential for using aAPCs nonspecifically to stimulate T cells for adoptive transfer.

To avoid microembolisms and antigenic reactions, a prerequisite for all non-cell, non-biodegradable aAPCs is the removal of all aAPCs before adoptive transfer. This goal has already been achieved for the above-described

approaches, by simply taking advantage of the magnetic properties of the beads.

Drosophila-based aAPCs (expressing HLA-A2, CD80 and CD54) (Figure 2c), as discussed above, have also been used to generate clinical-grade antigen-specific T cells against the melanoma antigen tyrosinase [74]. After an initial round of T-cell induction with the aAPCs, these were replaced by autologous APCs for two additional rounds of stimulation. A standard PCR did not detect any *Drosophila* antigens in any of the samples, suggesting a negligible risk of contamination of the T cells with xenoantigens. Shortly before infusion, the T cells were tested and found to be ~92% CD8⁺ and up to 10–30% tyrosinase specific. Ten patients with different stages of metastatic melanoma were enrolled in the study; none of the patients showed signs of toxicity after administering the T cells. When the distribution of the transferred T cells was monitored, homing to the tumor lesions, which had been reported with DC-stimulated T cells [34], could not be detected, and T cells found in biopsies of lesions were not clearly antigen specific [74].

Although, as summarized above, there are interesting studies on the use of aAPC-stimulated CTLs for adoptive transfer, several points concerning safety and efficacy remain to be studied more intensively. These include: (i) antigenic specificity of the infused T cells; (ii) reproducible manufacturing procedures; (iii) culture conditions, particularly sterility; and (iv) functional issues (e.g. division, homing, memory and lytic capabilities). Some of these issues are already met by the approaches discussed above, whereas others are being actively investigated.

Reproducible manufacturing of the aAPCs is a crucial point because a system used in a clinical setting must be reliable in a large-scale system. As such, approaches developed by Sadelein *et al.* [59–61] and the magnetic-bead-based aAPCs established by Oelke *et al.* [53,66] are interesting alternatives to the cell-based approaches described above. Both approaches are extremely promising in terms of their ability to induce antigen-specific T cells of the effector memory phenotype effectively. This leads to the potential that these cells will be highly functional *in vivo*. Thus, whereas cell-based aAPC systems (Figure 2b,c) have shown promise clinically, standardization, storage and ease of use might make bead-based aAPC systems (Figure 2a,d,e,g) preferable in clinical settings. Bead-based systems could be used in large-scale production of antigen-specific T cells sufficient for use in clinical trials. Furthermore they meet all of the pivotal requirements listed in Box 1.

Concluding remarks

In summary, adoptive immunotherapy represents an approach with great potential for the treatment of cancer and infectious diseases, especially in the setting of an immunosuppressed patient. Clinical studies performed by several groups have shown that adoptive immunotherapy induces only minimal or controllable side-effects [23,24,28,31,34,75]. Some of the studies even show remarkable benefit for the patients. Still, there are major impediments related to the reproducibility, functionality and generation of adoptively transferred

Box 2. Outstanding questions

- How many T cells are necessary for successful adoptive immunotherapy?
- Do infectious diseases require the same amounts of antigen-specific cells as cancer treatments?
- Is it of benefit or requirement to transfer antigen-specific cytotoxic T cells and antigen-specific helper T cells together?
- Can we alter the affinity of T cells and therefore their efficacy clinically using different aAPC formulations?

T cells that highlight the need for new approaches. A potential solution could be the use of some of the aAPCs summarized in this review. The technological advances described in this article, along with other methods, have brought remarkable progress to the field of immunotherapy, resulting in promising clinical trials. It is clear that adoptive immunotherapy has enormous potential as supportive therapy against cancer and infectious diseases. Initial clinical trials should be performed by transferring antigen-specific CTLs for the treatment of immunosuppressed bone marrow recipients to prevent viral infections. To explore the potential of aAPC-induced tumor-specific CTLs, one should apply adoptive T-cell transfer as an adjuvant therapy for malignant melanoma to eradicate minimal residual diseases and micrometastases after conventional therapy has been used to remove the main tumor.

We believe that the most promising aAPC is the HLA-Ig-based aAPC, which is supported by strong *in vitro* data [53,66]. Furthermore, it has been shown with the anti-CD3-based nonspecific aAPC that such a bead-based system is feasible and reliable for clinical applications and even has efficacy where well-defined antigen-specific T cells are not required [54]. In addition, possibilities exist for the development of class II HLA-Ig-based systems for the generation of antigen-specific CD4⁺ Th cells. This is especially interesting because, although their role in adoptive transfer is still relatively unexplored, it is clear that they are crucial for the development of robust immune responses and T-cell memory. Therefore, we are certain that these developments, together with further progress (Box 2), will lead to a new era of adoptive immunotherapy.

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