Review

T cell targeting in cancer therapy

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Summary. Targeting of immune cells by bispecific antibodies has proven a powerful tool for the investigation of cellular cytotoxicity, lymphocyte activation and induction of cytokine production, as well as to represent an innovative form of immunotherapy for the treatment of cancer. The hallmark of this approach is the use of the specificity of monoclonal antibodies to join target and immune cells by virtue of the dual specificity of bispecific antibodies for the two entities. More precisely the bispecific antibody has two different binding sites, which are capable of recognizing tumor associated antigens on the one hand and lymphocyte activation sites on the other. This process of crosslinking results in the activation of the lymphocyte and triggering of its lytic machinery, as well as lymphokine production. A major advantage of this therapeutic modality is, that use is made of the normal cellular immune defence system and therefore is only associated with minor toxicity. The distinct lymphocyte populations, which can be used for adoptive immunotherapy and the various bispecific antibody preparations, as well as the chimeric immunoglobulin/T cell receptor construction are the major topics of this review.

Key words: T lymphocytes – Bispecific antibody – Cancer therapy

Introduction

The vertebrate immune system can eliminate and neutralize virtually any foreign invader and distinguish such antigens from the body's own. To this end we possess two major classes of immune responses: the humoral and the cellular responses. The humoral response is mediated by antibodies that are produced by B lymphocytes upon specific antigen recognition. The binding of antibody to antigen facilitates the elimination of the invader by other immune cells. The cellular response involves specialized cells such as T lymphocytes and non-specific natural killer (NK) cells, monocytes, macrophages and granulocytes. The T lymphocyte population can be divided into two mutually exclusive subpopulations, i.e. TCRαβ and TCRγδ lymphocytes.

Experimentally induced tumour cells can express new antigens (Ag) on their surface. Such Ag are unique or selective and may be immunogenic and thus elicit a tumour-specific cytotoxic T lymphocyte (CTL) response. The study of tumour-specific immune reactions was stimulated by the postulation of immune surveillance as formulated by Ehrlich in 1909 [31], and adapted by Burnet in 1970 [23]. The keynote of the hypothesis is that antigen-specific T lymphocytes are the critical surveillance cells that arrest or eliminate cancer cells from the host. In general, specific T cells recognize their antigen in the context of the major histocompatibility complex (MHC) on the surface of the Ag-presenting cell. In experimentally induced tumour models such MHC-restricted tumour-specific T cells have been identified. In humans, "tumour-specific" CTL, which are not MHC-restricted, also have been demonstrated [22, 110].

Most efforts, however, to produce human CTL specific for human tumours have failed. Therefore the discovery that T cells can be recruited and targeted to a tumour cell by either bispecific antibodies (bs-mAb) or by equiping them with chimeric immunoglobulin/T cell receptors (Ig-TCR) has drawn considerable attention. These two strategies allow the focussing of immune effector cells to tumour cells and hold great promise for immunotherapy.

CTL/target cell interaction.

The interaction between a cytotoxic lymphocyte and a target cell can be divided into several stages [8, 67, 68]. The initial adhesion step is the rapid formation of an intercellular bond. Because CTL also bind to target cells that do not express the relevant MHC/antigen complex, and anti-TCR monoclonal antibodies (mAb) can not inhibit the

initial binding step, it was concluded that the initial interaction does not involve a specific TCR/antigen interaction [94]. Blocking studies with mAb against cell-surface molecules have identified two major pathways of antigen non-specific interactions [88]. First, leucocyte function-associated antigen-1 (LFA-1 or CD11/18 a) on the surface of the effector cell interacts with intercellular adhesion molecule (ICAM)-1 (CD54) or ICAM-2 [64, 65, 88, 96, 98]. Secondly, CD2 on the effector lymphocyte interacts with LFA-3 (CD 58) on the target cell [49, 87, 88]. When present on the surface of the cytotoxic lymphocyte, CD4 and CD8 may also contribute to this initial adhesion by binding to MHC class II or class I molecules respectively [83, 92, 93]. The initial adhesion phase is dependent on the presence of Mg^{2+} [88]. The second stage of the cytotoxic process consists of target cell recognition, which results in stabilization of the interaction between the effector- and the target cell, and leads to effector cell activation. Target cell recognition and activation of T cells occur through the TCR/CD3 complex, in which the TCR is responsible for the antigen recognition and the CD3 molecule for the transduction of signals generated by the TCR-antigen interaction [25].

Stabilization of the interaction between the effector cell and the target cell is not only due to the extra TCR-antigen bond. TCR crosslinking also results in a transient conformational change of the LFA-1 molecule. Consequently, the LFA-1 mediated adhesion temporarily increases [30, 52].

In fact, crosslinking of TCR/CD3 complexes at the cell-cell contact region also induces changes in the conformation and distribution of other cell surface molecules: TCR/CD3 crosslinking induces the redistribution of CD4 or CD8 molecules, which lead these molecules towards the contact region, where they become physically associated with the TCR/CD3 complexes [11, 55, 85]. The intracellular domains of CD4/CD8 antigens are also physically associated with the lymphocyte specific tyrosine protein kinase (TPK) p56lck. This TPK plays an important role in T cell activation, by phosphorylation of the ζ chain of the TCR/CD3 complex.

The CD4/CD8 molecules, that are physically associated with TCR/CD3 complexes, are believed to bind to the same MHC molecule that presents the antigen, leading to an increased CTL-target cell binding and optimal CTL activation [4, 85]. Taken together, it is clear that the co-clustering of TCR/CD3 with various other adhesion/signal transduction molecules at the cell-cell contact region, and their physical and functional interactions, critically control activation of the effector lymphocyte. The third stage of the cytotoxic process consists of the delivery of the lethal hit to the target cell and subsequent target cell death [114]. After delivery of the lethal hit, the effector lymphocyte dissociates from the target cell and may bind to another, a process that is referred to as recycling [67, 68].

TCRαβ and γδ lymphocytes

The availability of anti-TCR/CD3 mAb has facilitated the elucidation of the protein structure of this receptor complex [28, 44, 66].

The TCR+ lymphocyte population comprises of two distinct lymphocyte populations which are both capable of exerting cell-mediated cytotoxicity:
1. T lymphocytes that express an antigen-receptor, that is a disulphide-linked heterodimer composed of an α and a β protein: TCRαβ lymphocytes [15].
2. T lymphocytes with an antigen-receptor composed of a γ and a δ protein. The γ and δ chains can be either disulphide or nondisulphide linked: TCRγδ lymphocytes [16, 20].

The TCRα and β genes encode transmembrane glycoproteins whose sequences are homologous to those of Ig [45, 113]. Together, the TCRα and β chains determine the specificity of the TCRαβ lymphocytes [29, 84]. The TCR itself is thought not to be involved in signal transduction. In fact, the TCR molecules are non-covalently associated with the CD3 molecular complex, which represent the signal-transducing structure [19, 74, 90].

T lymphocytes can also be activated by anti-TCR/CD3 mAb. For activation to occur the mAb, and thereby the TCR/CD3 complexes, need to become crosslinked. Such Ab-induced T cell activation is polyclonal [61]. Crosslinking of the TCR/CD3 complex, either by antigen-specific stimulation or by anti-TCR/CD3 mAb, initiates signal transduction and leads to full T cell activation, expression of the interleukin-2 receptor (IL-2R), production of lymphokines, cytolytic activity, and proliferation. As described, most TCRαβ lymphocytes express either the CD4 or CD8 molecules. The helper/inducer functions are exhibited by CD4+ lymphocytes, whereas CD8+, and a minority of CD4+, lymphocytes exert cytolytic activities [36, 63, 77]. The antigen specific recognition by CD4+ lymphocytes is restricted by MHC class II antigens, whereas that of CD8+ lymphocytes is restricted by MHC class I molecules [101]. The CD4/CD8 glycoproteins play distinct functions in T cell activation. Again, they stabilize or increase the avidity of the interaction of the TCR/CD3 complex and the Ag-MHC by binding to the same MHC molecule that presents the antigen. However they also play an important role as an independent signal transducing element.

The cells of the minor TCRγδ lymphocyte fraction, (3%–4% of the TCR+ lymphocytes) virtually all lack the expression of CD4 or CD8 antigens [16, 53, 99, 106]. Freshly isolated TCRγδ lymphocytes do not lyse NK susceptible target cells. Upon in vitro activation, TCRγδ lymphocytes can exert MHC-unrestricted cytolysis towards a variety of tumor cells of distinct histologic origin, which does not involve the TCR. Antigen specific TCRγδ lymphocytes have also been identified. Some of the identified Og are CD1c, TCT-1, HLA-A2, A23, DR7 and Dw6 [95]. TCRγδ lymphocytes can be divided on the basis of their functional rearrangements into two distinct subsets. The Vγ9-Vδ2 encoded TCRγδ heterodimer is the predominant receptor type in peripheral blood, whereas the Vδ1 encoded TCRγδ heterodimer represents a minor population [99]. These distinct TCRγδ subpopulations show also distinct MHC unrestricted activity towards superantigens [35, 100]. Superantigens are defined as structures that activate a total lymphocyte population which uses one particular V gene element.

Natural killer cells

NK cells are lymphoid cells with typical "large granular lymphocyte" (LGL) morphology, and represent approximately 10% of peripheral blood lymphocytes (PBL). NK cells are functionally defined by their ability to display cytotoxicity against various target cells without prior activation [46, 75, 103]. They do not productively transcribe TCR genes [9, 58]. Therefore, their lytic activity is MHC-unrestricted. By definition, NK cells lack a memory response. In addition to their cytotoxic activity, a wide variety of non-cytotoxic functions have been identified, e. g. the production of a plethora of cytokines in response to stimulation with lectins, viruses, bacteria or NK susceptible target cells. The lymphokines produced by NK cells include interferonα and interferonγ (IFNα, IFNγ), IL-1, IL-2, natural killer cytotoxic factor, lymphotoxin and colony stimulation factor (CSF). The capacity to produce this plethora of lymphokines illustrates their important immunoregulatory role for NK cells [2, 80, 104, 112].

NK cells commonly express CD2, CD16 and NKHI (CD56) membrane antigens [81]. In contrast to TCR$\alpha\beta$ lymphocytes, single anti-CD2 mAb preparations can induce cytotoxicity by NK cells in some target cell combinations, indicating that the CD2 alternative activation pathway is also functional in NK cells [14, 89]. The CD16 (FcγRIII) molecule represents the low-affinity receptor for the Fc fragment of IgG, and is involved in antibody dependent cellular cytotoxicity (ADCC). The ability to mediate ADCC via CD16 points to a signal-transducing capacity of CD16 [79, 105, 111]. In NK cells the ζ chain is expressed in association with the CD16 molecule on the membrane and appears necessary for efficient surface expression of CD16 [5, 6, 59].

Non-TCR surface receptors involved in MHC-unrestricted cytolysis

The putative receptors involved in MHC-unrestricted cytotoxicity have not as yet been identified. It has been suggested that multiple receptors are involved in target cell recognition and MHC-unrestricted cytotoxicity: the multireceptor model [47, 69]. In this model, lysis of target cells by activated lymphocytes can be triggered by the interplay of signals transduced by several cell-surface molecules that interact with their respective ligands on the target cell. The interactions between these molecules on the effector lymphocyte and the relative expression of their ligands on the target cell together determine whether or not the target cell will be lysed by the effector cell [12, 15, 47].

The CD2 molecules serve as a functionally important interaction structure on TCR$\alpha\beta$, TCR$\gamma\delta$ and NK lymphocytes. As detailed earlier, CD2 mediates the adhesion of the effector to the target cell via binding to its natural ligand, i.e. LFA-3 or CD58, thereby contributing to antigen non-specific lymphocyte activation [96]. Three functionally important epitopes, T11.1, T11.2 and T11.3, have been identified on CD2 [70]. Only combinations of mAb directed against the CD2 epitopes T11.2 and T11.3 can induce IL-2 dependent $\alpha\beta$ T cell proliferation; provide help for antibody responses, and induce antigen non-specific cytolytic activity by CTL [14, 54, 109]. Single anti-CD2 mAb usually block $\alpha\beta$ T cell activation and proliferation [10, 18]. Recently it has been shown that upon T cell activation via CD2, the CD2 is not only functionally but also physically associated with the CD3 molecular complex [21, 72].

Other nonpolymorphic glycoproteins, such as CD3, CD16, and CD28, are also involved in the lymphocyte activation and triggering for lysis. Some of these structures are functionally interrelated, or they interact with the TCR$\alpha\beta$, TCR$\gamma\delta$ or putative NK lymphocyte receptor and with each other. This functional interplay between distinct activation sites suggests that their individual activation pathways, converge intracellularly [3, 13, 72, 105]. All data thus strongly argue in favor of our previously published hypothesis that a multireceptor recognition process is involved in MHC-(un)restricted target-cell recognition and lysis [47]. Therefore, activation via one receptor can be enhanced via another using combinations of the relevant mAb [13, 14, 17, 39, 76, 105].

Gene organisation and protein structure of the T cell receptor and immunoglobulins

The structures present in the immunesystem, which are capable of specific antigen recognition are 1) the surface bound or secreted Ig on B cells and 2) the TCR expressed by T cells. The four distinct TCR chains $\alpha,\beta,\gamma,\delta$ form two different heterodimers ($\alpha\beta$ and $\gamma\delta$).

The genomic organization of the TCR elements resembles that of the immunoglobulin gene superfamily. Also their quaternary structure shows great similarity [28].

TCR and Ig genes

Like the Ig light chain loci, the TCR α and γ chain loci comprise a number of variable gene segments (V), joining gene segments (J) and one or more constant gene segments (C). The Ig heavy chain loci and the TCR β and γ chain loci comprise additional diversity gene segments (D) between the V and J clusters [28, 91]. Functional TCR or Ig genes are assembled in an identical way in a two-step process of recombination. First, fusion of particular V, (D) and J segments within the DNA, by deletion of intervening sequences, creates a complete V-coding domain upstream of the C gene segment(s). Second, after transcription of the entire gene, RNA-splicing enzymes splice the non-coding intron that separates the V(D)J and C gene segments. The resulting sequence of messenger RNA is translated into protein. Diversity of TCR and Ig is not only created by the joining of particular V, (D), J and C gene segments but also by the random addition of nucleotides at the V-(D)-J joining sites [57]. The contribution of somatic mutations on diversity is, however, specific for Ig [28].

TCR and Ig protein structures

Comparison of the primary sequence of TCR and Ig reveals that the residues that are principally responsible for the framework structure of the V regions are highly conserved [27]. This implies that TCR and Ig V regions fold in the same fashion, and that the chain pairing and the resulting combining sites of $V\alpha$-$V\beta$ and $V\gamma$-$V\delta$ are similar to those of V_H-V_L combinations. The loops that form the antigen binding site in TCR are similar in size and location to those found in Ig but have different conformations. Also the structures at the V-C interphase of TCR and Ig are very similar, indicating that the V and C domains of TCR and Ig fold in the same way. Recently it has been found that membrane-bound Ig are associated with two other transmembrane proteins, $IgM\alpha$ and $Ig\beta$, which form a disulphide-linked heterodimer [48]. In analogy to the TCR/CD3 relation, it was suggested that these proteins act as a signal transducer for the membrane bound Ig.

Targeting of lymphocyte specificity

Although most efforts to produce human CTL specific for human tumours have failed, many mAb are available that bind selectively, albeit not exclusively, to tumour cells. The combined use of CTL and tumour-selective mAb, offered a novel approach to target the CTL to the tumor cells selectively. These engineered lymphocyte specificities may in the future play a key role in the destruction of tumour cells. The employment of (a) mAb, (b) bs-mAb or (c) chimeric Ig-TCR receptors to engineer lymphocyte specificity for targeting to tumour cells together with cytokines represents a promising approach.

*Monoclonal antibody targeting of cytotoxic
T lymphocytes*

As earlier detailed, CTL can only lyse target cells that express the relevant Ag/MHC complex on their cell surface. However, CTL can also be induced to lyse target cells that they would normally not recognize and lyse [105]. This process is referred to as targeting of lymphocytes. For instance, anti-TCR/CD3 mAb can mimic the activation that occurs upon TCR-Ag/MHC recognition. Anti-TCR/CD3 mAb induces lysis of IgG-Fc-receptor-positive target cells because the mAb bridges the CTL and the target cell, and simultaneously activates the lytic machinery of the CTL [62, 105]. Also mAb directed against other activation sites, such as CD2, CD16 and CD26, can induce lysis of target cells [14, 37, 38, 105].

Bispecific mAb targeting of cytotoxic T lymphocytes

An interesting exploitation of the phenomenon that mAb mediated lymphocyte activation results in triggering of the lytic machinery (see above) is the use of bs-mAb [78, 97]. Bs-mAb can be produced by chemically coupling a mAb that is specific for, e.g. the TCR complex to a second mAb,

for example one specific for a given tumour-cell-associated antigen. Also chemically crosslinked antibodies have been produced with one binding site recognizing the CD16 activation site on NK cells and the other recognizing a tumour-associated antigen [50]. Such mAb heteroaggregates bridge the effector lymphocyte to the target cell (conjugate formation) and trigger the lymphocyte lytic machinery. In the latter example the heteroconjugated antibody triggers lymphocyte-mediated cytolysis by binding to CD16 via its Fab, rather than via its Fc portion.

Along the same line, many preparations of bs-mAb, that recognize the CD3 complex on the one hand and a tumour-associated antigen on the other (e.g. melanoma, renal, ovarian, lung and mammary carcinoma) have been developed [24, 60, 102, 106, 107]. Such bs-mAb have also been generated by fusion of two hybridomas that produce CD3/TCR-specific mAb or the target-cell-specific mAb respectively [71]. The hallmark of bs-mAb targeting is that the binding of the bs-mAb to the, by their nature uniquely, specific CTL now targets the CTL with the bs-mAb specificity. These bs-mAb-targeted CTL can effectively be used for adoptive transfer of immunity [86].

Since mAb with specificity for particular lymphocyte activation sites exist that trigger different functions or lymphocyte subpopulations, their selective use allows the activation and expansion of particular subsets of lymphocytes and eliminates the need for prior isolation of these lymphocyte subsets. These laboratory-targeted immune lymphocytes are presently being used for locoregional clinical adoptive immunotherapy. We and others have recently started a phase I-II clinical trial employing bs-mAb-retargeted lymphocytes for the intraperitoneal treatment of ovarian carcinoma patients [73].

Chimeric immunoglobulin-T cell receptor

Bs-mAb-targeted T lymphocytes retain the antibody-dictated specificity only for limited periods of time (6–72 h), due to the dissociation of the bs-mAb from the cell surface [60] (Bolhuis, manuscript in preparation). In addition, it is not known whether the bs-mAb targeted lymphocytes can traffic, and eventually home to the tumour site. Thus, the bs-mAb targeted T lymphocytes may at this stage be therapeutically most effective when applied locoregionally. The systemic eradication of primary tumours and metastasis requires "long-lasting" and systemic immune responses. Molecular engineering of T lymphocytes can result in such permanently acquired, laboratory chosen, tumour selectivity of T lymphocytes, i.e. by the construction of chimeric Ig-TCR receptors. To become effective, such T lymphocytes require not only the stable expression of the engineered Ig-TCR receptor at the lymphocyte surface, but also its functional association with the CD3 signal-transducing elements. This condition was met by the introduction and expression of chimeric Ig/TCR genes, in which the variable (V) gene segments of the $TCR\alpha$ and $TCR\beta$ chains were replaced by the variable gene segments of the heavy and light chain of an Ig with known specificity [7, 32, 41–43].

Transfection of the expression vectors containing these chimeric Ig/TCR genes in recipient mouse T cell lines resulted in the synthesis of chimeric proteins which became stably and functionally expressed at the lymphocyte surface of the lymphocytes. Both combinations of complementary chimeric Ig/TCR genes, i.e. $V_H-C\alpha + V_L-C\beta$ or $V_H-C\beta + V_L-C\alpha$, appeared to yield a functional chimeric heterodimer [43, 56]. Consequently, the chimeric receptor equiped the T lymphocytes with the antibody specificity which, of course, is MHC-unrestricted. These chimeric receptors were able to transduce signals for T cell activation, as determined by Ca_i^{2+} mobilization [56]; IL-2 production [32, 40–43]; proliferative response [40], and cytotoxic activity [40, 42, 43].

Relatively large numbers of lymphocytes expressing the chimeric receptor are required for therapeutic applications. In order to reduce the need for prolonged lymphocyte expansion, it is important that a high percentage of lymphocytes can simultaneously be infected. Such a high multiplicity of infection also ensures that the genetically modified T cells are polyclonal. This may be best achieved by retrovirus-mediated gene transfer, which can transduce a wide variety of cell types with a much higher efficiency than other procedures [26]. Another advantage of retrovirus-mediated gene transfer is that all proviral copies become stably integrated into the chromosomal DNA of the recipient cell. This chromosomal integration of the transduced gene guarantees the constitutive expression of chimeric Ig/TCR genes, a prerequisite for prolonged immune reactivity. Meanwhile, mouse and human T lymphocytes have been successfully transduced with a number of genes using retroviral vectors [1, 26, 33, 34, 51, 82].

Before clinical application of these genetically modified lymphocytes takes place we have to know whether the administration of these lymphocytes is safe. After all, foreign DNA is inserted randomly in the genome and this may theoretically initiate an oncogenic transformation. However, the results obtained in vitro in animals and those obtained after and during 56 months of observation of patients who received Neo-gene transfected autologous T lymphocytes in a phase I clinical protocol, have not revealed any abnormalities or side-effects.

Concluding remarks

The targeting of immune effector cells to tumour cells by bs-mAb or by chimeric Ig-TCR receptors offers potential clinical applications. Many questions still need to be answered:

Is the triggering for cytolysis by CTL important or is the induction of lymphokine production by CTL or non-CTL critical?

Can the targeted CTL traffic and home like physiologically activated T cells? Does lymphokine production at the tumour site cause "bystander" tumour cell lysis which prevents the escape of those tumour cells that lack the relevant tumour antigen expression?

Does "humanization" of the mouse mAb prevent or reduce human anti-mouse Ab responses that may block their therapeutic effect?

What are the optimal time schedules for administration of adoptive immunotherapy?

Data addressing these different questions are emerging. However, we should not delay clinical studies until all the questions have been answered, provided of course that the trials fulfil the accepted criteria of good laboratory and clinical practice.

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