Treatment of Hodgkin’s disease with bispecific antibodies

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Summary

Bispecific monoclonal antibodies (Bi-MAbs) with dual specificity for tumor-associated antigens (TAA) and a triggering molecule of an immunologic effector cell, respectively, open the possibility to specifically target to and activate cytotoxic effector cells (macrophages, T-cells, NK cells) at the tumor site. Using appropriately designed Bi-MAbs and unstimulated human NK cells and T-cells, respectively, we were able to cure SCID mice xenografted with human Hodgkin’s tumors. This approach was also effective in disseminated tumors and when treatment was delayed until three weeks after the inoculation of the tumor, thus establishing this approach as the most effective model of an immunomodulating therapy of human neoplasms. Early observations with an ongoing phase I/II study with CD16/CD30 Bi-MAb in patients with refractory Hodgkin’s disease confirm the expected low toxicity. If these observations can be confirmed in larger clinical studies, effector cell activating Bi-MAbs could become an important weapon in the remaining fight for the conquest of Hodgkin’s disease.

Key words: bispecific antibodies, Hodgkin’s disease, immunotherapy

Introduction

While immunotherapeutic approaches have been demonstrated to be quite successful in animal models, immunotherapy of human neoplasms has so far met with only limited success. While treatment of human cancer with native monoclonal antibodies (MAbs) or even toxin- or radionuclide-conjugated MAbs was generally well-tolerated with no or few side-effects, the clinical success of MAbs has so far been quite limited (for review see [1]). There might be a small therapeutic window for such approaches in cases with a low tumor burden, as has been suggested by the results of a trial with monoclonal antibody 17-1 in the adjuvant (post-operative) setting of human colorectal carcinoma [2]. To overcome this problem, an approach is needed, which combines the specificity of monoclonal antibodies with the high cytotoxicity of certain immune effector cells and thus should be capable to eliminate a clinically measurable tumor [3]. Bispecific monoclonal antibodies (Bi-MAb) that have a dual specificity for both tumor targets and immune cells have the potential to combine the advantages of antibody specificity with the cytotoxic capabilities of effector cells [4]. Besides their ability to enrich effector cells at the tumor site, Bi-MAbs can make an additional contribution to the immune recruitment by their potential to activate tumor-bound effector cells. This is possible if the Bi-MAb arm which binds the effector cell is directed against and able to activate trigger molecules expressed on the surface of these cells. Since the monovalent binding of one arm of the Bi-MAb is insufficient to trigger immune effector cells, efficient activation will occur only at the tumor site, where Bi-MAbs mediate crosslinking between effector and target cells, but not at other encounters of effector cells with Bi-MAbs [5, 6]. Therefore, side effects observed after the systemic administration of cytokines which are caused by circulating activated effector cells should not be a limitation for the clinical application of Bi-MAbs.

Production of Bi-MAbs

Bi-MAbs can be obtained in different ways (for review see [7]): First, so-called heteroconjugates are obtained by the chemical crosslinking of two monoclonal antibodies with distinct specificities; and, second, hybrid Bi-MAbs are obtained from hybrid hybridomas which are established by somatic fusion of two hybridomas secreting the proper antibodies [4].

Selection of triggering molecules on effector cells

Several effector cells of the natural and specific immune system can mediate cellular cytotoxicity after appropriate stimulation: phagocytic cells, natural killer (NK)-cells and T-lymphocytes (for review see [8]). All these cells express specific trigger molecules which activate the cytolytic machinery after binding of the appropriate ligand (Table 1). The natural ligand of these triggering molecules can be substituted for by a stimulatory antibody.
Phagocytic cells can execute target cell specific cytotoxicity via binding and activation of the phagocytic cell's Fc receptors to the Fc proportion of an antigen-specific antibody. The most widely used FcyRIIAB mediated targeting of phagocytes is the FcyRII (CD64). Most in vitro data on phagocytic cell-binding and -activating Bi-MAbs were obtained from studies in infectious diseases, in particular HIV infection. A recently published study which employed a FcyRI specific Bi-MAb for targeting G-CSF pre-activated granulocytes against breast cancer tumor cells has shown that this approach can also be applied to the experimental treatment of malignancies [9].

**Table 1. Cytotoxic effector cells and their triggering molecules.**

<table>
<thead>
<tr>
<th>Surface triggering molecules</th>
<th>Cellular distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD2</td>
<td>T-cells, NK cells</td>
</tr>
<tr>
<td>CD3</td>
<td>T-cells</td>
</tr>
<tr>
<td>T-Cell Receptor (TCR)</td>
<td>T-cells</td>
</tr>
<tr>
<td>CD16 (FcyRII)</td>
<td>NK cells, macrophages;</td>
</tr>
<tr>
<td></td>
<td>T-cell subpopulations, neutrophils</td>
</tr>
<tr>
<td>CD28</td>
<td>T-cells</td>
</tr>
<tr>
<td>CD32</td>
<td>Monocytes, macrophages, neutrophils</td>
</tr>
<tr>
<td>CD64 (FcyRI)</td>
<td>Monocytes, macrophages; neutrophils</td>
</tr>
<tr>
<td></td>
<td>after stimulation with IFN-γ and G-CSF</td>
</tr>
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</table>

**Treatment of Hodgkin's disease with NK cell activating Bi-MAbs**

NK-cells are cytotoxic lymphocytes that, in the absence of prior stimulation, lyse a variety of target cells and probably represent the first line of unspecific (non-MHC-restricted) cellular defense mechanisms. The majority of freshly isolated or cultured tumor cells are relatively resistant to the activity of unstimulated peripheral blood NK cells and they become susceptible to NK-cell lysis only following NK-cell activation by culture in IL-2 (LAK cells). Unlike conventional cytotoxic T-lymphocytes, cytotoxicity of NK cells is not restricted by MHC gene products. NK cells lack the CD3/T-cell receptor complex and express CD56 and CD16 (Fcy-receptor III) surface markers. An important pathway of NK-cell activation is mediated by the CD16 (FcyRIII receptor) surface molecule.

In our study of Bi-MAb mediated NK cell lysis for Hodgkin's disease, we selected the CD16 [5] MAb-producing cell line A9 as a partner for HRS-3 which produces antibodies against the CD30 molecule which is highly expressed on Hodgkin and Reed–Sternberg cells. As a preclinical model for the evaluation of human NK-cell mediated tumoricidity in vivo, we used severe combined immunodeficiency (SCID) mice, which have no functional T- and B-cells. These mice do not reject xenografts of human lymphocytes or human tumors and can be repopulated with human lymphocytes. Subcutaneously growing Hodgkin's cell tumors were established in SCID mice and grown to a size of 4 to 6 mm diameter. Tumor-bearing animals received 100 μg of Bi-MAb HRS-3/A9 together with 10^7 unstimulated human peripheral blood lymphocytes. Complete remission of the tumor was achieved in all animals treated this way. Sixty percent of the animals, however, had relapsing tumors. The cure rate of 40% achieved by a single administration of human PBL and NK-cell activating Bi-MAbs represented the most effective model of an immunomodulating therapy of established solid human tumors using human effector cells [5] and appeared to be significantly more effective than ricin-A-chain immunotoxins prepared from the same antibody [10]. Moreover, it seems possible to increase the cure rate of established human xenografted tumors using NK-cell activating Bi-MAbs by the concurrent application of interleukin-12 [11].

**Treatment of Hodgkin's disease with T-cell activating Bi-MAbs**

A third effector cell system which can be targeted to and activated at the tumor site by Bi-MAbs are T-lymphocytes. In addition to T-cell receptor (TCR) recognition of antigen (Ag) presented in the context of a MHC-molecule by an antigen presenting cell (APC) costimulatory signals are needed for T-cell proliferation or effector function. Without costimulation, exposure of T-cells to an antigen may cause unresponsiveness and clonal anergy or deletion. Costimulation can be provided by a variety of cytokines (e.g., IL-2) or membrane bound molecules, most of which belong to the group of adhesion molecules, e.g. members of the B7-family (B7-1, B7-2 and B7-3), which represent the natural ligands for the CD28 and CTLA-4 counter-receptors on T-cells.

To determine the in vivo efficacy of an anti-tumor approach that uses only Bi-MAbs for activation and targeting of the effector T-cells, we took advantage of the possibility of stimulating resting T-cells by combined triggering of the TCR complex and CD28. A combination of purified CD3/CD30 and CD28/CD30 Bi-MAbs derived from tetradosomas induced a significantly stronger proliferation of CD3+ cells in monocyte- and natural killer cell-depleted human peripheral blood lymphocytes in the presence of CD30+ Hodgkin's derived L540 cells than did a combination of the parental monospecific MAbs to CD3, CD28, and CD30 or either of the Bi-MAbs alone [6]. Tumor cell lysis was clearly antigen dependent and not MHC-restricted as only CD30+ positive tumor cells or COS cells (which lack human MHC molecules) transfected with the cDNA coding for the CD30 antigen can serve as targets for Bi-MAb activated lymphocytes [13].

We could show that T-lymphocytes activated by antigen crosslinked by Bi-MAbs enter the cell cycle, upregulate the expression of a variety of activation markers (e.g., CD25, Ki-67) and proliferate rapidly
depending on the respective T-lymphocyte subset. CD4+ lymphocytes were the most rapidly expanding subpopulation after Bi-MAb stimulation, but Bi-MAb-directed cytotoxicity was mediated mostly by CD8+ lymphocytes and effector cells belonging to the CD45RO+ pool. Blocking of the LFA-1/ICAM-1 or CD2/LFA-3 adhesion pathway decreased Bi-MAb mediated cytotoxicity, which was not due to inhibition of aggregate formation between Bi-MAb coated T-lymphocytes and target cells. Additional CD2 cross-linking resulted in tyrosine phosphorylation of distinct cellular substrates. These findings indicate that adhesion molecules function as costimulatory signals rather than as cellular contact mediators in T-lymphocytes stimulated by Bi-MAbs which enhance signal transduction through the CD3/TCR and CD28 pathways [12]. T-lymphocytes from patients suffering from Hodgkin's disease could be activated as effectively as the lymphocytes of healthy donors and conferred a strong cytotoxicity against autologous tumor cells or established tumor cell lines.

Having defined the conditions for the induction of Bi-MAb mediated T-cell cytotoxicity in vitro, we treated SCID mice bearing established human L540CY Hodgkin's derived tumors of 6 to 8 mm in diameter by injection of Bi-MAbs CD3/CD30 and CD28/CD30 or the respective parental antibodies through the tail vein. Tumor-bearing animals received 1 × 107 previously stimulated human PBLs intravenously 24 hours after administration of the antibodies. All animals (two series of 10 animals in each treatment group) that had received the mixture of Bi-MAbs CD3/CD30 and CD28/CD30 were cured of the xenografted human tumors. All other animals had progressing tumors and were killed by day 40. The combination of Bi-MAbs with reactivity to CD3 and CD28 and a TAA, respectively, represents the most effective model for the treatment of xenografted human tumors to date. We were able to demonstrate that human lymphocytes activated by the combination of Bi-MAbs with specificity for CD3 and CD28 and a tumor-associated antigen are only found in tumor tissue, and to a much lesser extent or not at all in tumor-antigen negative tissues or unrelated tumors established in the same mice [14]. Moreover, we showed that the majority of activated lymphocytes stays in TAA positive tissue and does not enter circulation [14]. Most importantly, only human T-lymphocytes obtained from the tumors of appropriately treated mice had detectable levels of perforin, granzyme A and granzyme B mRNA.

Meanwhile, we could show that due to the long persistence of Bi-MAb at the tumor in vivo, in vitro stimulation of the T-cells is not necessary. Moreover, this model is also effective in curing SCID mice from disseminated Hodgkin's tumors [15]. These two observations are important, as they suggest the efficacy of a simplified modification of this approach (i.e., without in vitro prestimulation) in the clinical situation.

The described experiments form the rationale for CD3 and CD28 Bi-MAb-based clinical trials of human cancers. The results obtained in SCID-mice are encouraging, especially if one considers that even mice suffering from disseminated tumor growth could be cured. A major advantage of the combined CD3/CD28 Bi-MAb approach compared to other immunotherapeutic strategies is the possibility to achieve a tumor-site specific activation of the T-cell cytolytic machinery. As the Bi-MAb targeted and activated lymphocytes do not home in TAA negative tissues, it can be hoped that the level of side effects of such a treatment should be low.

**Phase I/II study with an anti-CD16/CD30 Bi-MAb in patients with refractory Hodgkin's disease**

Part of the attraction of the immunotherapeutic approach with NK-cell activating Bi-MAbs is founded in its simplicity which makes it comparable easy to test this approach in the clinical situation. Indeed, after we had succeeded in producing sufficient amounts of pure Bi-MAbs from the A9/HRS-3 hybrid hybridoma cell line, a phase I/II clinical trial of relapsed Hodgkin's disease has been initiated in July 1995, which by the time of this meeting is still ongoing. Eligible for this study were patients with Hodgkin's disease who fulfilled the following criteria: (1) >2nd relapse, which was refractory to ≥2 chemotherapy regimens; not eligible for high-dose chemotherapy; not eligible for curative radiotherapy; (2) Age: 18–60 years; (3) no chemoradiotherapy within 4 weeks prior to Bi-MAb; (4) progressive disease with measurable tumor parameters; and (5) life expectancy >4 months. The treatment consisted of an 1-hour i.v. infusion of the CD16/CD30 Bi-MAb times four every three to four days. The dose escalation scheme is shown in Table 2. To date, 11 patients have been treated, all in stage IV, with lung and bone marrow being the predominant organ involvement. The mean number of preceding chemotherapy regimens was 3 (range 2 to 7) with five patients relapsing after high-dose chemotherapy with autologous bone marrow or peripheral blood stem cell support. Most patients had also received radiotherapy.

**Toxicity:** Despite of the reduced performance status of the patients, treatment has so far (up to a dose of 16

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Day 1 (mg/m²)</th>
<th>Day 4 (mg/m²)</th>
<th>Day 8 (mg/m²)</th>
<th>Day 11 (mg/m²)</th>
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<td>1</td>
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<td>7</td>
<td>64</td>
<td>64</td>
<td>64</td>
<td>128</td>
</tr>
</tbody>
</table>
mg/m²) been well tolerated and the maximal tolerated dose has not been reached. Fever and light pain in affected lymph nodes was observed in two patients, an allergic rash after the fourth infusion in one. Human anti-mouse immunoglobulin antibodies (HAMA) have been detected in 1/4 patients tested.

A final evaluation of the anti-tumor efficacy of the Bi-MAb in this ongoing trial is to be expected soon.

**Perspectives for the use of Bi-MAbs in clinical trials**

The major obstacles for the use of Bi-MAbs in clinical trials to date are the difficulties in producing Bi-MAbs in sufficient quantity and quality needed for administration in patients, as well as the HAMA (human-anti-mouse-antibody) response against applied Bi-MAbs. Recently, Kostelny and coworkers [16] described a new, genetic method for the production of bispecific antibody fragments using heterodimer-forming sequences which ensure efficient production of the bispecific molecules. As to the second problem of murine Bi-MAbs, the induction of HAMAs, the strategy described by Holliger and coworkers [17] seems to be very promising. They established so-called ‘diabodies’ by linking the V₅ and V₅ of two different antibodies A and B to form two different ‘cross-over’ chains V₅-A-V₅-B and V₅-B-V₅-A. Because of their size (50 kDa) and the absence of an Fc fragment, the biodistribution of these recombinant bispecific peptides should be superior to Bi-MAbs established by the tetradoma technique.

In summary, the specific local activation of the immune system at the tumor site by recombinant bispecific antibody fragments promises to become an effective tool for treating minimal residual disease with a choice of different effector systems. There is reasonable hope that such novel approaches will improve upon the promising results which were recently reported for native MAbs in the adjuvant treatment of colorectal carcinomas and establish Bi-MAbs as an effective treatment of clinically manifest tumors [2].

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**References**