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Immune recruitment by bispecific antibodies for the treatment of Hodgkin disease

Abstract For the treatment of Hodgkin lymphoma, bispecific monoclonal antibodies (bi-mAbs) were established which recognize the Hodgkin-associated CD30 antigen with one arm and the CD3 or CD28 antigen on T lymphocytes or the CD16 antigen on natural killer (NK) cells with the second arm. The NK cell-activating α-CD16/CD30 antibody was able to retarget human NK cells toward $CD30^+$ target cells and induce their lysis. Sixty percent of Hodgkin tumor-bearing severe combined immunodeficient mice responded to a combined treatment with bi-mAb and human NK cells, leading to a final cure rate of 20%. T cell-activating bi-mAbs were more effective, resulting in the cure of all mice treated. The in vivo administration of both α-CD3/CD30 and α-CD28/CD30 antibodies resulted in the specific activation of resting human T cells infiltrating the $CD30^+$ Hodgkin tumors. Tumor-infiltrating lymphocytes in the group of mice treated with both T cell-activating bi-mAbs expressed high levels of cytokines and cytotoxic molecules such as perforin and the cytotoxic serine esterases granzyme A and B. More importantly, activated T cells did not home to $CD30^-$ tissue and did not enter the circulation. Encouraged by these preclinical data, 15 patients with treatment-refractory Hodgkin lymphoma were included in a phase I/II dose-escalation study and treated four times every 3 or 4 days with increasing doses of the α-CD16/CD30 bi-mAb ranging from 1 mg/m^2 to 128 mg/m^2. No dose-limiting toxicity occurred even at the highest doses. Of these 15 patients, one had a complete response, one a partial response, three a mixed response, two stable disease, and eight patients had progressive disease. Treatment with immunological effector cell-recruiting bi-mAbs is a promising new approach to the treatment of Hodgkin disease refractory to standard therapy.

Key words Immune recruitment · Biospecific antibodies · Hodgkin disease

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Introduction

Among many immunotherapeutic approaches for the treatment of malignant disease, monoclonal antibodies (mAbs) were one of the first for which efficacy was confirmed in clinical trials. The successful treatment of colorectal cancer [10] in an adjuvant setting or relapsed low-grade lymphoma [8] with antibodies demonstrated the clinical potential of therapeutic strategies that are capable of redirecting humoral and cellular effector functions to tumor cells. However, the major obstacle to a more general use of mAbs is their variable and usually low cytotoxic potential. Therefore alternative concepts were developed over the past few years that take advantage of the high tumor specificity of mAbs for specific target antigens [13]. Within this group of new treatment concepts, bispecific monoclonal antibodies (bi-mAbs) have proven their efficacy in many tumor systems in vitro and in preclinical studies with animal models. The advantage of bi-mAbs is their ability to bridge tumor and effector cells together and induce local cell destruction. Immunological effector cells that can be activated by such bi-mAbs include granulocytes, macrophages, natural killer (NK) cells, and T cells [3, 5, 12].

NK cells are genuine killer cells with prestored cytoplasmic granula that contain cytotoxic molecules such as perforin and granzymes [4]. These molecules are rapidly released when appropriate trigger receptors expressed on the membrane of NK cells (e.g., CD16) are crosslinked by an antibody. In contrast, T cells need two mitogenic stimuli to be activated and exert their cytotoxic effector function. Most commonly, a combination of two bi-mAbs specific for a tumor-associated antigen (TAA) and the CD3 and CD28 antigen on T lymphocytes, respectively, is used to activate resting T cells fully [9]. Over the past few years, we have generated one NK cell- (targeting the CD16 antigen) and two T cell- (targeting the CD3 and CD28 antigens, repectively) activating bi-mAbs for the treatment of Hodgkin lymphoma. We used the CD30 antigen on Hodgkin-Reed-Sternberg cells as a target since biodistribution trials in patients had revealed excellent targeting properties.

Materials and methods

Antibodies and cell lines

The generation, purification, and characterization of the bi-mAbs α-CD16/CD30, α-CD3/CD30, and α-CD28/CD30 have been described previously [9]. The $CD30^+$ human Hodgkin-derived cell lines L540CY and HDLM2, lymphoblastoid cell lines Daudi and Raji, and the erythroleukemic cell line K562 have been described elsewhere [2, 11].

NK and T cell preparation

Peripheral blood mononuclear cells (PBMCs) from heparinized blood of healthy donors were isolated and NK or T cells were negatively enriched by magnetic-activated cell sorting. Contaminating cell fractions were always less than 0.5%.

Fluorescence-activated cell sorting analysis

Single-cell suspensions were obtained by mechanical dissection of different organs. Five thousand human lymphocytes were analyzed in each experiment. For intracellular staining of interleukin (IL)-1β, IL-2, and tumor necrosis factor (TNF)-α, cells were permeabilized with 0.5% saponin (Sigma, Munich, Germany). An FITC-conjugated goat anti-mouse F(ab′) immunoglobulin (Ig) monomer antibody was used as the second-step reagent (Dianova, Hamburg, Germany). For visualizing membrane-bound T cell activation markers Ki-67, CD25, CD45RO, CD3, CD4, and CD8, a double-immunofluorescence assay was performed using either two primarily FITC- or PE-conjugated mAbs.

Severe combined immunodeficient mouse tumor model

Pathogen-free, 4- to 6-week-old severe combined immunodeficient (SCID) mice (C.B-17 lcr SCID/SCID) were obtained from the Institut für Versuchstierzucht (Hannover, Germany). Animals were housed and bred in laminar flow racks and fed with autoclaved standard chow and water. "Leaky" animals were identified by enzyme-linked immunosorbent assay (ELISA) and excluded from further experiments. The experimental model was based on the solid growth of two different tumors in the same mice. L540CY cells 2×10^7 cells were subcutaneously injected into the left ventral thoracic wall, and the $CD30^-$ LOVO cell line was injected subcutaneously into the right ventrogluteal region of the same animal. When tumors reached a diameter of 1 cm, bi-mAbs followed by resting human T lymphocytes were injected in the ventral or dorsal tail vein. There were four experimental groups (four to ten mice per group): 1) the $CD3^+$ lymphocyte treatment group (1×10^7 T cells, suspended in phosphate-buffered saline [PBS] at a volume of 300 μL); 2) $CD3^+$ lymphocyte and 2F10-bi-mAb (α-CD28/CD30) 100 μg treatment group; 3) $CD3^+$ lymphocyte and HT3-bi-mAb (α-CD3/CD30) 100 μg treatment group; and 4) $CD3^+$ lymphocyte and two bi-mAb (α-CD3/CD30 and α-CD28/CD30) 50 μg treatment group. Seven days after administration of the bi-mAbs and effector cells, the mice were killed by cervical dislocation. The solid tumors, spleens, and blood were removed for further investigation.

Clinical trial in patients with Hodgkin disease

Patients were eligible if they had histologically confirmed $CD30^+$ Hodgkin disease at second or more relapse or were refractory to at least two standard polychemotherapy protocols and not curable by radiotherapy alone. They had to have been off treatment (including radio-, chemo- or immunotherapy) for at least 4 weeks with measurable tumor parameters. The bi-mAb HRS-3/A9 was produced under good medical practice conditions by Biotest Pharma GmbH (Dreieich, Germany) and contained >95% intact murine IgG-1 antibody. One treatment cycle consisted of four bi-mAb infusions given intravenously every 3–4 days over 1 h. The initial dose was 1 mg/m^2. Two patients were treated at each dose level and the dose was doubled for the next two patients if no severe side effects (US National Cancer Institute Committee on Common Toxicity Criteria (CTC) grade 3 or 4) occurred until the maximum dose of 64 mg/m^2 was reached. Toxicity and response criteria were evaluated as described [7]. Blood counts with differentiation and analysis of circulating lymphocyte subsets were performed before and after the end of the bi-mAb infusion as well as 1, 3, 6, 24, 48, and 72 h thereafter. The human anti-mouse antibody (HAMA) response to HRS-3/A9 was assayed by ELISA. Blood samples were collected at the indicated time points, and PBMCs were isolated by Ficoll gradient separation and stored at −80 °C until the end of the study. At this stage, NK cell activity was measured using a standard 2-h EuCl cytotoxicity assay. K562 (NK sensitive) and Daudi tumor cells (NK resistant) were used as target cells at an effector:target ratio of 20:1. All experiments were performed in triplicate.

Results

Generation and characterization of NK cell-activating bi-mAbs

We had previously generated a variety of high-affinity anti-CD30 antibodies and confirmed in a small clinical trial that the one antibody tested (HRS-3) targeted Hodgkin lymphoma tissue in patients. Therefore we combined this antibody with a newly established anti-CD16 antibody to generate an NK cell-activating bi-mAb. The final antibody was able to bridge human NK cells and $CD30^+$ tumor cells together in a concentration-dependent fashion. In addition, the binding of the bi-mAb to the CD16 antigen activated resting NK cells and initiated tumor cell-specific destruction. Hodgkin tumors xenotransplanted into SCID mice were successfully treated with a combination of peripheral blood

lymphocytes and this new bi-mAb. Sixty percent of all animals treated were cured by single bi-mAb administration, confirming the efficacy of this approach for the treatment of Hodgkin lymphoma.

Generation and characterization of T cell-activating bi-mAbs

A combination of two bi-mAbs recognizing the CD30 antigen with one arm and the CD3 or CD28 antigen on T cells with the other was needed to achieve antigen-specific, but major histocompatibility class-unrestricted T cell activation. Using Hodgkin lymphoma as a model, we demonstrated that bridging of Hodgkin tumor cells to T cell-triggering molecules with a combined α-CD30/CD3 and α-CD30/CD28 bi-mAb regimen induced efficient tumor cell lysis in vitro and in vivo. In a preclinical model, treatment of SCID mice harboring xenografted disseminated $CD30^+$ tumors resulted in the complete cure of all animals. The in vivo administration of both α-CD3/CD30 and α-CD28/CD30 bi-mAb resulted in the specific activation of xenotransplanted resting human T cells infiltrating the $CD30^+$ Hodgkin tumor. Bi-mAb treatment enhanced the expression levels of cytokines such as interleukin-1β, interleukin-2, TNF-α, and activation markers including Ki-67, CD25, and CD45RO in tumor-infiltrating lymphocytes. This antigen-dependent local T cell stimulation led to the activation of the cytolytic machinery in T lymphocytes, as demonstrated by the upregulation of mRNA coding for perforin and the cytotoxic serine esterases granzyme A and B. Bi-mAb-induced generation of cytotoxic T lymphocytes was strictly correlated with the presence of the CD30 antigen and the combined application of both bi-mAbs.

Phase I/II clinical trial with an NK cell-activating bi-mAb in patients with Hodgkin disease

Although our preclinical data suggested that T cell-activating bi-mAbs might be more effective than NK cell-activating ones, we decided for logistic reasons to study the NK cell system in a clinical setting first. A total of 15 patients were included in the first phase I/II clinical trial. All patients were extensively pretreated and presented with stage IV disease. The majority (eight of 15) had a history of extended-field radiotherapy, and seven had undergone high-dose chemotherapy with autologous bone marrow transplantation or peripheral blood stem cell support, and the remainder had not been eligible for high-dose chemotherapy and stem cell support due to the failure to harvest sufficient numbers of stem cells or to medical reasons that precluded this aggressive treatment. The antibody treatment was well tolerated, with the maximum tolerated dose not reached at 64 mg/m^2 given four times. Mild and moderate side effects (CTC grade I and II only) were seen in six patients. There was no correlation between bi-mAb dose and type or severity of side effects. Repeated treatment after 4 weeks could not be performed because of allergic reactions. The response rate (one complete response, one partial response, and three mixed responses in 15 patients) in our trial with the NK cell-activating HRS-3/A9 bi-mAb was surprisingly high and lasted from 1 to 18 months.

Following bi-mAb infusion, no consistent changes in peripheral blood counts of any subpopulation including NK cells and monocytes were observed. Nine patients (60%) developed HAMAs blocking the binding of the bi-mAb with its antigen. At the cellular level, bi-mAb doses higher than 4 mg/m^2 induced a significant increase in cytotoxic NK cell activity which lasted for up to 6 weeks after treatment.

Discussion

In the present report, we provide evidence that bi-mAbs can be used for the treatment of Hodgkin lymphoma. Retargeting either NK or T cells by bi-mAbs resulted in the efficient lysis of tumor cells in vitro and in vivo. A phase I/II clinical trial with the NK cell-activating bi-mAb showed promising results with a respone rate of 30%. In contrast to the results of other groups [14], treatment with our bi-mAb was well tolerated and we did not reach the dose-limiting toxicity even at the highest dose of 64 mg/m^2. However, repetitive treatment was not possible because all patients developed allergic reactions after reexposure, a phenomenon that is well known with the use of antibodies of murine origin [1]. Our results should encourage further clinical trials with this novel immunotherapeutic approach and emphasize the necessity to reduce the immunogenicity of the antibody to allow retreatment of responding patients. Nevertheless, in a second clinical trial, we changed the treatment schedule and compared bolus versus continuous infusion to test the hypothesis that antibodies given at constant levels are less immunogenic. All patients entering the second trial could be treated for multiple cycles, with only two patients developing low HAMA levels without any clinical signs of allergic reactions.

Our preclinical data suggest that T cell-activating bi-mAbs might be more effective and underline the major advantage of the combined α-CD3-/α-CD28 bi-mAb approach by inducing a tumor site-specific activation of the T cell cytolytic molecular program in vivo. As bi-mAb-targeted and -activated lymphocytes did not home to TAA-negative tissues, the extent of side effects caused, e.g., by damage to normal tissue by activated T cells, should be low.

Even more important, recent studies in syngeneic mouse models proved the induction of tumor-specific T cell clones after treatment with bi-mAbs [6]. If this observation holds true in human beings, treatment with T cell-activating bi-mAbs might be a simple way to initiate an active immune response. Therefore T cell-targeting bi-mAbs might have a dual function: first, to activate a large pool of resting T cells randomly within days; and

second, to initiate the induction and expansion of a tumor-specific T cell clone with long-term activity. Pending clinical trials with this approach will show whether such a combined passive and active dual immunotherapeutic approach will result in the cure of a significant proportion of patients who would succumb to their disease with standard therapy.

References

1. Clark JI, Alpaugh RK, von Mehren M, Schultz J, Gralow JR, Cheever MA, Ring DB, Weiner LM (1997) Induction of multiple anti-c-erbB-2 specificities accompanies a classical idiotypic cascade following 2B1 bispecific monoclonal antibody treatment. Cancer Immunol Immunother 44: 265
2. Diehl V, von Kalle C, Fonatsch C, Tesch H, Juecker M, Schaadt M (1990) The cell of origin in Hodgkin's disease. Semin Oncol 17: 660
3. Fanger MW, Graziano RF, Guyre PM (1994) Production and use of anti-FcR bispecific antibodies. Immunomethods 4: 72
4. Ferrini S, Prigione I, Miotti S, Ciccone E, Cantoni C, Chen Q, Colnaghi MI, Moretta L (1991) Bispecific monoclonal antibodies directed to CD16 and to a tumor-associated antigen induce target-cell lysis by resting NK cells and by a subset of NK clones. Int J Cancer 48: 227
5. Garrido MA, Perez P, Titus JA, Valdayo MJ, Winkler DF, Barbieri SA, Wunderlich JR, Segal DM (1990) Targeted cytotoxic cells in human peripheral blood lymphocytes. J Immunol 144: 2891
6. Grosse-Hovest L, Brandl M, Dohlsten M, Kalland T, Wilmanns W, Jung G (1999) Tumor-growth inhibition with bispecific antibody fragments in a syngeneic mouse melanoma model: the role of targeted T-cell co-stimulation via CD28. Int J Cancer 80: 138
7. National Cancer Institute (1993) Investigator's handbook. A manual for participants in clinical trials of investigational agents. Cancer therapy evaluation program, DCT, National Cancer Institute, Bethesda, MD
8. McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK (1998) Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 16: 2825
9. Renner C, Pfreundschuh M (1995) Tumor therapy by immune recruitment with bispecific antibodies. Immunol Rev 145: 179
10. Riethmueller G, Holz E, Schlimok G, Schmiegel W, Raab R, Hoffken K, Gruber R, Funke I, Pichlmaier H, Hirche H, Buggisch P, Witte J, Pichlmayr R (1998) Monoclonal antibody therapy for resected Dukes' C colorectal cancer: seven-year outcome of a multicenter randomized trial. J Clin Oncol 16: 1788
11. Sahin U, Kraft-Bauer S, Ohnesorge S, Pfreundschuh M, Renner C (1996) Interleukin 12 increases bispecific-antibody-mediated natural killer cell cytotoxicity against human tumors. Cancer Immunol Immunother 42: 9
12. Segal DM, Qian JH, Mezzanzanica D, Garrido MA, Titus JA, Andrew SM, George AJ, Jost CR, Perez P, Wunderlich JR (1992) Targeting of anti-tumor responses with bispecific antibodies. Immunobiology 185: 390
13. Staerz UD, Yewdell JW, Bevan MJ (1987) Hybrid antibody-mediated lysis of virus-infected cells. Eur J Immunol 17: 571
14. Weiner LM, Clark JI, Davey M, Li WS, Garcia de Palazzo I, Ring DB, Alpaugh RK (1995) Phase I trial of 2B1, a bispecific monoclonal antibody targeting c-erbB-2 and Fc gamma RIII. Cancer Res 55: 4586