Initiation of humoral and cellular immune responses in patients with refractory Hodgkin’s disease by treatment with an anti-CD16/CD30 bispecific antibody

Abstract Fifteen patients with refractory Hodgkin’s disease were treated in a dose-escalation trial with the bispecific monoclonal antibody (bi-mAb) HRS-3/A9, which is directed against the Fcγ receptor III (CD16 antigen) and the Hodgkin’s-associated CD30 antigen. Treatment consisted of four cycles of four bi-mAb infusions given over 1 h every 3–4 days at different dose levels ranging from 1 mg/m² to 64 mg/m². Measurable serum levels (above 0.1 µg/ml) of circulating bi-mAb could be detected in patients treated with doses above 4 mg/m², reaching peak levels of 9.5 µg/ml immediately after the end of antibody infusion on the highest dose level. Bi-mAb elimination corresponded to second-order kinetics with a terminal half-life time ($t_{1/2}$) of 28–32 h.

Bi-mAb treatment induced the occurrence of human anti-(mouse Ig) antibodies (HAMA) in 6 out of 13 patients initially testing negative. All 6 patients not only developed anti-isotypic anti-(mouse Ig) but also anti-idiotypic and anti-anti-idiotypic antibodies. While no consistent changes of peripheral blood cell counts, or of any lymphocyte subpopulation including natural killer (NK) cells, has been observed, 4 out of 6 evaluable patients treated with doses of at least 4 mg/m² showed an increase of NK cell activity within 2 weeks after treatment, which lasted for a maximum of 12 weeks. Circulating amounts of soluble CD30 antigen could be detected in the serum of 6 patients. However, like the results and time courses of all the other immunological parameters evaluated, this was not predictive for treatment outcome.

Key words Immunotherapy · Monoclonal antibodies · Hodgkin’s lymphoma

Introduction

Among many immunotherapeutic approaches to malignant diseases, monoclonal antibodies (mAb) were one of the first to have their efficacy confirmed in clinical trials. The success of treating colorectal cancers [26] in an adjuvant setting, or relapsed low-grade lymphoma [17] with antibodies, demonstrates the clinical potential of therapeutic strategies that redirect humoral and cellular effector functions to tumor cells. The major handicap of native monoclonal antibodies is their variable and usually low cytotoxic potential. Therefore, concepts have been developed that take advantage of the high tumor specificity of mAb for the specific delivery of cytotoxic drugs, enzymes or radioisotopes [7, 27]. In addition, antibodies have been generated that are able to redirect and activate potential cytotoxic effector cells to the tumor site [31]. This concept of bispecific antibodies (bi-mAb) has proven its efficacy in multiple tumor systems in vitro and in preclinical studies with animal models. Immunological effector cells that can be activated by such bi-mAb include granulocytes, macrophages, natural killer (NK), and T cells [9, 11, 29]. In contrast to T cells, NK cells are genuine killer cells with pre-stored cytoplasmic granula that contain cytotoxic molecules such as perforin and granzymes [10]. These molecules are rapidly released when appropriate trigger receptors, expressed on the membrane of NK cells (e.g. CD16), are crosslinked by an antibody [21]. The advantage of bi-mAb is their ability to bridge tumor and effector cells together and induce local cell destruction. Although this concept has been known for many years and the number of papers on this topic is quite high, there are only two reports of clinical trials with
NK-cell-activating bi-mAb [13, 34]. This is mostly because of problems inherent to the production of sufficient amounts of antibody of clinical-grade quality.

In our study [13, 25], we evaluated an NK-cell-activating CD16/CD30 bi-mAb (HRS-3/A9) in patients with Hodgkin’s disease refractory to (high-dose) chemotherapy and radiotherapy. In vitro and in vivo experiments using Hodgkin’s-derived tumor cell lines heterotransplanted into SCID (severe combined immunodeficiency) mice demonstrated that the CD16/CD30 bi-mAb was very effective in redirecting human NK-cell-mediated lysis to CD30+ Hodgkin’s cells [15]. Despite the fact that the patients who were entered into the subsequent phase I/II study presented with absolute lymphocyte numbers characteristic for end-stage Hodgkin’s patients, 30% of the heavily pretreated patients responded to therapy, and remissions lasting up to 15 months were observed [13]. Repeated treatment of responding patients was not possible as all patients showed allergic reactions when a second bi-mAb application was tried 4 weeks after the initial treatment.

To gain further insights into possible immunological effects induced by the treatment with CD16/CD30 bi-mAb, we analyzed in more detail the pharmacokinetics of the antibody and the generation of human anti-(mouse Ig) antibodies (HAMA). In addition, we performed NK cell assays with frozen peripheral blood lymphocytes to test NK cell activity at different times during and after treatment. As nothing is known about the evolution of NK cell activity during and after treatment with NK-cell-activating bi-mAb, a careful characterization of these parameters could suggest important improvements of the infusion regimen, which should ultimately lead to an increased treatment efficacy with higher and longer-lasting remissions.

**Patients and methods**

**Patient and treatment schedule**

The patient characteristics have been described extensively before [13]. Altogether, 15 patients with progressive Hodgkin’s disease, who were refractory to standard treatment procedures, entered the phase I/II clinical trial and were eligible for analysis. All patients gave written informed consent. Patients with negative delayed-type hypersensitivity (DTH) after bi-mAb exposure were treated with four HRS-3/A9 infusions given intravenously in 250 ml 5% human albumin solution over 1 h every 3–4 h. The starting dose was 1 mg/m² for each infusion. Two patients were treated at each dose level, and the dose was doubled for the next 2 patients if no side-effects of CTC grade 3 or 4 were observed until the maximum dose (because of the limited amount of bi-mAb available) of 64 mg/m² was reached.

**Cell lines and fusion proteins**

The CD30+ human Hodgkin’s-derived cell lines (L540CY and HDLM2), lymphoblastoid cell lines (Daudi, Raji) and the erythroleukemic K562 cell line have been described elsewhere [5, 22]. All cell lines were cultured in RPMI-1640 medium (Gibco, Karlsruhe, Germany), supplemented with 10% (v/v) fetal calf serum, 2 mM glutamine, streptomycin (100 mg/ml) and penicillin (100 IU/ml). A fusion protein (CD30-Ig), consisting of the extracellular CD30 domain and a murine IgG1 Fc fragment, was generated in our laboratory [22]. The cell lysate from CD30-positive (L540CY) and negative cell lines (HPB-ALL) was extracted as previously described [12].

**Antibodies**

The generation, purification and characterization of the parental anti-CD30 (HRS-3) and anti-CD16 (A9) antibodies and of the bispecific anti-CD16/CD30 antibody have been described previously [15]. For the clinical study, the bispecific antibody has been produced under conditions of good manufacturing practice by Biotest Pharma GmbH (Dreieich, Germany) and contained more than 95% intact murine IgG1 bi-mAb. The mAb used in this study for fluorescence-activated cell sorting (FACScan) analysis were phycoerythrin(PE)-labeled anti-CD3, fluorescence-isothiocyanate(FITC)-labeled anti-CD14, anti-HLA-DR-PE, anti-CD16-FITC, anti-CD19-PE and FITC-labeled goat anti-(mouse Ig) serum (Becton Dickinson, Heidelberg, Germany). Polyclonal goat anti-(mouse Ig) serum (unconjugated and peroxidase (POX) conjugated) and polyclonal biotinylated goat anti-[human F(ab)2] were all purchased from Dianova (Hamburg, Germany).

**Isolation of peripheral blood mononuclear cells (PBMC)**

PBMC were isolated by Ficoll-Hypaque (Pharmacia, Sweden) density gradient centrifugation as described before [22]. Macrophages were removed by plastic adherence (37 °C, 1 h)

**Blood count analysis and FACScan analysis**

Blood counts, with differentiation (Coulter STKS) and analysis of circulating lymphocyte subsets (FACScan, Becton Dickinson), were performed before and after the end of the bi-mAb infusion as well as 1, 3, 6, 24, 48, 72 h thereafter. FACScan analysis with directly labeled antibodies or FITC-conjugated goat anti-(mouse Ig) serum was performed as previously described [13, 22].

**Determination of antibody levels and the induction of anti-idiotypic antibodies**

Levels of circulating bispecific antibody were assessed by a sandwich enzyme-linked immunosorbent assay (ELISA). In brief, ELISA plates were pre-coated with goat anti-(mouse Ig) serum (dilution 1:2000, 4 °C, overnight) blocked with 1.5% gelatin in TRIS-buffered saline and the patient’s serum was added in serial dilutions. A purified murine antibody of the IgG1k subclass was used as standard. Binding of bispecific antibodies was detected by a peroxidase-labeled goat anti-(mouse Ig) serum (dilution 1:5000, room temperature, 1 h). Human anti-(mouse Ig) antibody (HAMA) response to HRS-3/A9 was assayed as described previously [13] with minor modifications. Briefly, ELISA plates were coated with bi-mAb HRS-3/A9 (2 µg/ml, 50 µl/well) overnight at 4 °C; non-specific binding was blocked by 1.5% gelatin (w/v) in phosphate-buffered saline at room temperature; dilutions of patients’ sera were incubated for 1 h at room temperature; after washing, 50 µl/well of a 1:1000 diluted biotinylated goat anti-[human F(ab)2] was added as the secondary antibody for 1 h at room temperature. After extensive washing, a 1:50 000 dilution of alkaline-phosphatase-conjugated streptavidin (Boehringer Mannheim, Germany) was incubated for 15 min at room temperature (100 µl/well) and, after additional washing, the reaction product was developed with nitrophenyl phosphate (Sigma, Munich, Germany) as substrate, stopped with HCl, and read at 405 nm on an ELISA reader (Dynatech MR 4000). All patients’ sera were pre-incubated (1 h, 37 °C) with 5 µg/ml irrelevant IgG1 control antibody [28] to absorb anti-idiotypic antibodies.