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Purging of Bone Marrow with a Cocktail of Monoclonal Antibodies (VIB-pool) for Autologous Transplantation *

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A. Introduction

Children with relapsed common acute lymphoblastic leukemia (ALL), B-non-Hodgkin lymphoma (B-NHL), and B-acute lymphocytic leukemia (B-ALL) have a very poor prognosis. If there is no HLA-identical donor, the patient should be considered for autologous bone marrow transplantation. Collected autologous marrow should be cleansed of contaminating tumor cells without destroying hemopoietic stem cells. We report here the results of our investigations with a cocktail of three monoclonal antibodies (VIL-A 1, VIB-C 5, and VIB-E 3 – the VIB pool – kindly given to us by Dr. Knapp of Vienna) concerning in vitro cytotoxicity against ALL blast cells and leukemic cells lines, lytic capacity with rabbit and human complement, and stem cell toxicity, together with our findings concerning the ex vivo purging of the marrow of four children. The antibodies have the particular advantage of being able to lyse blast cells of common ALL-type and B cells very effectively with human complement [5].

B. Material and Methods

I. Antibodies

The VIB pool used is a cocktail consisting of three monoclonal antibodies of the IgM type (VIL-A 1, VIB-C 5, and VIB-E 3) and reacts with the common ALL antigen (CALLA) – VIL-A 1 – and with two different epitopes of the CD 24 surface structure – VIB-C 5 and VIB-E 3.

II. Complement

We used as complement human AB serum and selected rabbit serum batches not toxic against human hemopoietic stem cells, and autologous serum for the ex vivo purging.

III. Cells

Target cells were fresh or cryopreserved leukemic cells from children with ALL, hemopoietic cell lines Reh and Nalm, and mononucleated bone marrow cells from healthy adult volunteers.

IV. Treatment of Cell Mixtures

The cotoxicity of the antibodies for the target cells was tested by using the trypan blue exclusion test and the specific ^{51}Cr-release.

V. Purging Protocol

Bone marrow cells from four children (three Burkitt-type NHL, stage III, and one common ALL) were harvested from the anterior and posterior iliac crest, and anticoagulated with preservative-free heparin. Mononuclear cells were isolated on Ficoll-Amido-

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trizoate gradients and resuspended at a concentration of $1-2 \times 10^7$ cells/ml. They were incubated with the antibody cocktail (30 µg/ml) at room temperature for 20 min. This procedure was followed by a twice-repeated 30-min treatment with autologous serum (final concentration 50%). The cells were cryopreserved in RPMI medium containing 5% dimethyl sulfoxide, 20% human albumin, and 20% autologous serum.

VI. Colony-Forming Assay

The enumeration of CFU-GM by a modified technique of Irvine et al. (1984) [2] was done in all patients at each stage of the investigation: (a) on the fresh marrow; (b) after incubation before freezing; and (c) after thawing.

C. Results and Discussion

1. It was possible to demonstrate a high lytic capacity of the antibody cocktail (VIB pool) with complement against CALLA-positive leukemic cells, blast cells of a child with B-ALL, and Nalm and Reh cells. There was no difference in the lytic capacity, whether rabbit serum or human AB serum was used as complement source (Fig. 1).
2. Lysis of CALLA-positive leukemic cells was detectable at antibody concentrations of as low as 0.08 µg/ml. The percentage of lysed cells ranged from 82% to 100% with leukemic cells and amounted to 100% with Reh cells (Fig. 2).
3. Treatment with the VIB pool and rabbit or human complement did not cause a significant loss of CFU-GM. The loss of CFU-GM due to cryopreservation amounted to $15 \pm 9\%$ (Fig. 3).
4. After the ex vivo purging of remission marrow of four children (3 B-NHL, 1 common ALL) with the VIB pool and human complement for autologous

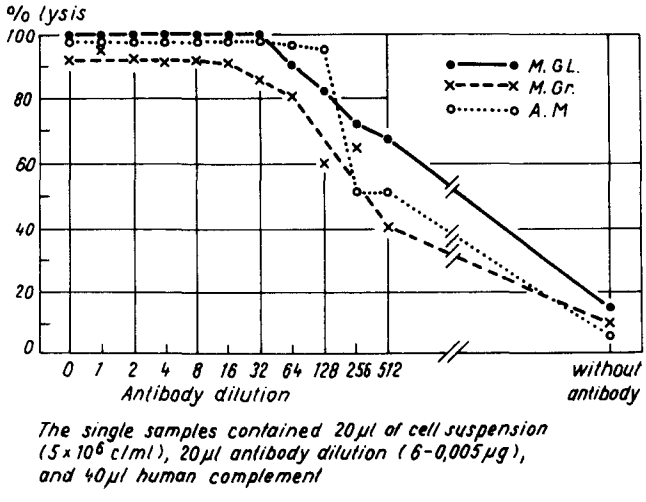

Fig. 1. Reactivity of the VIB pool against different target cells

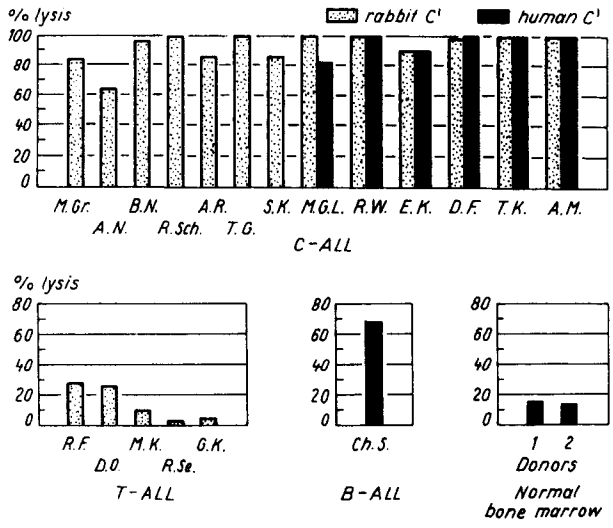

Fig. 2. Reactivity of the VIB antibody cocktail with CALLA$^+$ ALL cells

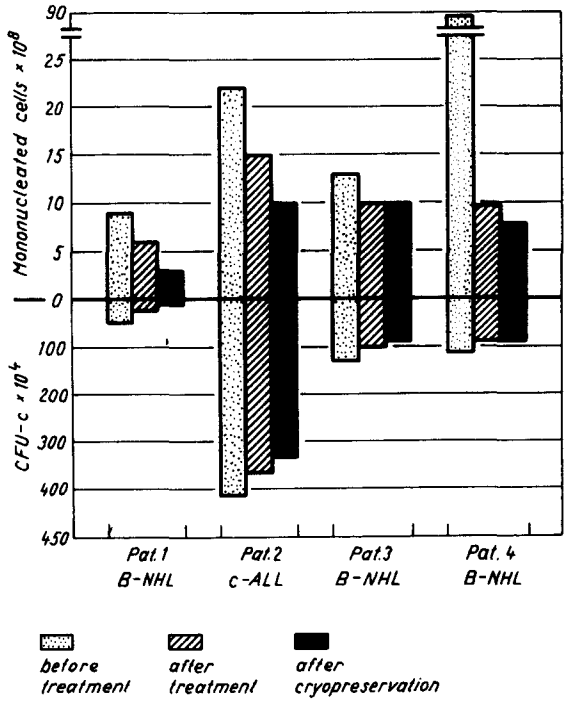

Fig. 3. Mononucleated cells and CFU-GM in bone marrow of four patients after in vitro purging with VIB pool and human complement

transplantation, there was a sufficient recovery of CFU-GM for cryopreservation ($2.2–10 \times 10^4$ CFU-GM/kg).

According to different investigations, an in vitro purging procedure with a combination of several monoclonal antibodies is more effective than with only one antibody [1]. All three components of the VIB pool are able to lyse leukemic cells of the common ALL type. The VIB-C 5 and VIB-E 3 components react with leukemic cells of the B-ALL type [3]. All three components are able to lyse CALLA ALL cells with human complement, as was shown by Sugita et al. (1986) [5] and ourselves. Compared to other monoclonal antibodies used for purging bone marrow [4, 1], which, as a rule, react with heterologous complement only, the VIB pool has the advantage of binding the rabbit and human complement. This results in a number of advantages for clinical application.

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