The Potential Usefulness of Interleukin-2 Activated Bone Marrow Cells as an Active Therapeutic Tool Against Cytomegalovirus Infection in a Bone Marrow Transplantation Setting

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Bone marrow transplantation (BMT) has been used in recent years for the treatment of immunodeficiency diseases, aplastic anemia, and leukemia. However, there are a number of serious problems and limitations associated with autologous or allogeneic BMT. One of these is an increase in opportunistic infections, of which cytomegalovirus (CMV) infection is one of the most important. Cytomegalovirus has been associated with more frequent deaths than any other single agent, with no reproducibly successful or therapy currently available. Recently usage of interleukin-2 or immunomodulation has been suggested as a powerful modality to combat infectious disease. In this study we showed that bone marrow activated in interleukin-2 for 2 days has the ability to lyse spleen cells infected for 3 days with murine CMV (acute infection model) or salivary gland cells infected for 7 days (chronic infection model), while nonactivated bone marrow or natural killer (NK) cells showed no such lysis. The majority of activated cells involved in lysis were anti-asialo GM₁⁻, Thy-1⁺, indicating a population of cells other than the natural killer-cell population involved.

KEY WORDS: Interleukin-2; bone marrow; cytomegalovirus.

INTRODUCTION

Bone marrow transplantation (BMT) has been used increasingly over the past few years for the treatment of immunodeficiency diseases, aplastic anemia, leukemia, and rare disorders of hemato-

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killer (NK)-resistant, fresh, autologous or syngeneic tumor cells in a short-term \(^{51}\text{Cr}\) release assay (5–10). These cells have been called lymphokine-activated killer (LAK) cells and their lytic specificity, kinetic properties, precursor, and effector cell surface phenotype have been characterized in both the human and the murine system (5–10). Recently, IL-2 therapy is also emerging as a promising treatment for a variety of infectious diseases (11–13) where there has been shown to be a significant reduction in viral titer/parasitic load in infected subjects treated with IL-2 (11, 12).

The potential of bone marrow cells as immunotherapeutic modality using biomodulation of marrow cells has not been completely studied in transplantation. This is one of the first studies of its type evaluating the efficacy of biomodulation of bone marrow as an active therapeutic tool against cytomegalovirus infection. The ability of IL-2-activated bone marrow cells to lyse cells infected with cytomegalovirus cell was assessed in both acute and chronic stages of cytomegalovirus infection in the murine system. There is no animal model for human cytomegalovirus (HCMV) but murine cytomegalovirus (MCMV) has many similarities in characteristics to human CMV (14). Therefore the murine cytomegalovirus model was used in this study and the effects of the activation of bone marrow in interleukin-2 on the reconstitution of mice were assessed.

METHODS

Virus and Tissue Culture

The Smith strain of murine cytomegalovirus (MCMV) was obtained from the American Type Culture Collection (ATCC, Rockville, MD) and introduced into either tissue culture using mouse embryo fibroblast (MEF) cells for \(\text{in vitro}\) passage, or BABL/c mice for \(\text{in vivo}\) viral passage as described below. The tissue culture MEF infected with murine cytomegalovirus was observed for viral cytopathic effects for 14 days.

Animals

BALB/c female mice 3 weeks of age were obtained from Jackson Laboratories (Bar Harbor, ME) and MCMV was given to mice intraperitoneally at \(5 \times 10^4\) plaque-forming units (PFU) per mouse using the method described below. Three-week-old mice were used in order to obtain more productive infections. The virus produces an acute infection during the first few days in which a variety of organs in peritoneal cavity is infected with the virus. The virus eventually produces a chronic infection in the mice with a high titer of virus remaining only in the salivary gland for several months. The MCMV spleen was harvested 3 days postinfection, made into a single-cell suspension using a cell homogenizer, and used for the “acute infection model” of virus. For the “chronic infection model” the MCMV-infected salivary gland was harvested 7 days post-viral infection, made into a single-cell suspension, and subsequently used (15). The spleen and salivary gland cells had a viability of over 95%. The infected spleen cells had an average MCMV virus titer of \(5 \times 10^4\) PFU/ml. The infected gland had an average MCMV virus titer of \(1 \times 10^5\) PFU/ml.

Plaque-Forming Assay

A plaque-forming assay for human CMV (DJ Lay, personal communication) will be adopted for use with MCMV. The plaque assay was done according to Chalmer et al. (16). Briefly, petri dishes seeded with MEF were infected with different known dilutions of MCMV, overlayed with 2% methylcellulose (Sigma, St. Louis, MO), incubated for 5 days at 37°C, stained with methylene blue (Sigma, St. Louis, MO), and plaque counted to establish a standard curve. The PFU assay was used to estimate the titer of MCMV used for animal injection.

Preparation of Interleukin-2-Activated Bone Marrow Cells

The interleukin-2 (IL-2) used was recombinant generated from Cetus Corporation (Emeryville, CA). Bone marrow was obtained from uninfected syngeneic animals, and the cells were flushed out of the femurs and tibias of animals using a 23-gauge needle, resuspended, and then washed using complete medium (CM) containing RPMI-1640 (GIBCO, Grand Island, NY) with 0.1 mM nonessential amino acids and 1 mM sodium pyruvate (Microbiological Associates, Walkersville, MD), 5 \(\times 10^5\) M 2-mercaptoethanol, 100 \(\mu\)g/ml streptomycin, 100 \(\mu\)g/ml penicillin, 0.03% glutamine, and 10% heat-inactivated fetal calf serum (GIBCO, Grand Island, NY). The cell suspension was passed.