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Glutathione Augments the Activation of Cytotoxic T Lymphocytes *in vivo*

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Abstract

The activation of cytotoxic T lymphocytes (CTL) *in vivo* was found to be augmented by glutathione if injected i.p. in the late phase but not in the early phase of the response. The effect of glutathione possibly resembles the augmenting effect of 2-mercaptoethanol in lymphocyte cultures.

Introduction

A variety of sulfhydryl compounds augment the activation of cytotoxic T lymphocytes in mixed lymphocyte cultures (1, 2) and the DNA synthesis in response to mitogens (3, 4). 2-mercaptoethanol (2-ME) is now routinely added to mixed lymphocyte cultures. The mechanism of this augmenting effect is still not completely understood. ROSENSTREICH and MIZEL (5) suggested that 2-ME enhances the stimulating effect of a macrophage product (TAF), and OPITZ et al. (6, 7) suggested that 2-ME activates a serum component. HOCHFELD and OPPENHEIM (8, 9) suggested, in contrast, that 2-ME may augment T cell responses by converting oxidized glutathione in the serum into reduced glutathione which in turn may remove the inhibitory effect of oxidized materials from the culture. Reports from two other laboratories indicated that 2-ME may inactivate a suppressor factor (10, 11). Another group of authors finally provided evidence that 2-ME acts directly on T and B lymphocytes and promotes their proliferation and differentiation (12–14). It has been reported that 2-ME facilitates the uptake of cystine from the medium by the lymphocytes (15).

In view of the potentiating effects of sulfhydryl compounds *in vitro*, it was of interest to investigate whether T cell responses *in vivo* may also be augmented. Since 2-ME is relatively toxic *in vivo*, we investigated the

effects of the more physiological compound glutathione (GSH) on T cell responses *in vivo* and *in vitro*. Glutahtione is a natural product and is in fact the most abundant sulfhydryl compound in higher organisms. It has also been shown (16) that the depletion of the intracellular pool of glutathione inhibits the activation (blast transformation) of lymphocytes.

Materials and Methods

Animals

Mice were obtained from the central breeding stock of the German Cancer Research Center, Heidelberg, F.R.G., or were purchased from Bomholtgard, Ry, Denmark.

The induction and assay of cytotoxic T cell responses in vivo

3 mg of cyclophosphamide (Endoxan, ASTA, Brackwede, F.R.G.) were injected i.p. into C3H mice 2 days before immunization (17–19). Immunization was performed by skin painting with 6 × 10 µl of a 30 % solution of TNCB in acetone on the four footpads and on two abdominal sites. The mice were sacrificed usually 5 days later, and their pooled axillary and inguinal lymph node cells were directly tested for cytotoxic activity against trinitrophenylated syngeneic target cells in a 4-h ^{51}Cr release assay in cultures with 3×10^{-5} M 2-ME as described elsewhere (20).

Results

Cytotoxic responses *in vivo* were induced in C3H mice by skin painting with TNCB as described in «Materials and Methods». The cytotoxic activity of the pooled axillary and inguinal lymph nodes against trinitrophenylated syngeneic target cells was tested 5 days after immunization. Mice which had been treated with reduced glutathione 3 days after immunization expressed an approximately 5-fold greater cytotoxic activity than untreated mice (Fig. 1). In contrast, no augmentation was seen in mice which had been treated with glutathione on the day of immunization (Fig. 1). The dose-response curve revealed an optimal augmentation at a concentration of 2×10^{-1} M glutathione in 2 × 0.3 ml BSS per mouse i.p. on day 2 and day 3 after immunization (Fig. 2).

Discussion

Our experiments have demonstrated that the activation of cytotoxic T lymphocytes *in vivo* is markedly augmented by the injection of glutathione during the late phase of the response. Glutathione (4) and other sulfhydryl compounds (3, 4) have previously been shown to augment mitogen-induced T cell responses *in vitro*, but effects of glutathione on T cell responses *in vivo* have not been described previously. The effect may be related to the

immune-modulating effect of levamisole (2, 3, 5, 6-tetrahydro-6-phenylimidazole [2,1-b] thiazole) (21–29) which is possibly converted into a sulfhydryl compound *in vivo*.

We have chosen to study glutathione because it is a naturally occurring sulfhydryl compound. Glutathione is indeed the most abundant sulfhydryl compound in higher organisms and is released by muscle and liver cells (30). Glutathione is known to regulate the functions of many enzymes (31, 32),

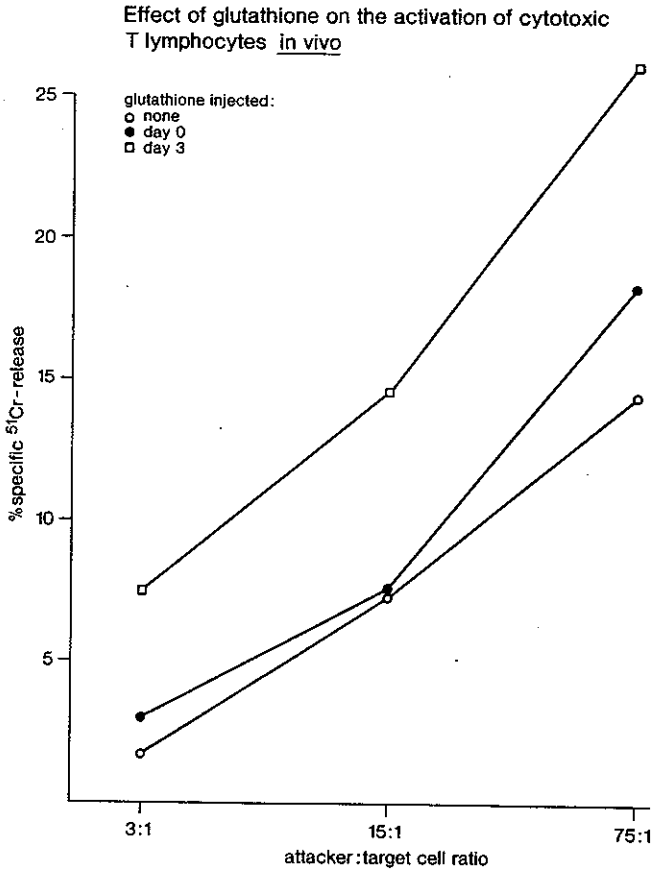

Fig. 1. Effect of glutathione on the activation of cytotoxic T lymphocytes in vivo. C3H Tif/Bom mice were treated with 3 mg cyclophosphamide i.p. and were immunized 2 days later by skin painting with 6 × 0.01 ml 20 % TNCB in acetone. The mice received also 0.2 ml 2 × 10^{-1} M reduced glutathione i.p. either on the day of immunization or 3 days later. The cells of the pooled axillary and inguinal lymph nodes were tested after 5 days in a 4-h ^{51}Cr release assay with concanavalin A-activated and trinitrophenylated C3H spleen as target cells. The injection of glutathione on day 3 mediated a 5-fold augmentation of the cytotoxic activity (lytic units), i.e., similar cytotoxic activities were obtained with 5-fold smaller numbers of attacker cells from glutathione treated mice.

and the depletion of the intracellular pool of glutathione was shown to inhibit the activation (blast transformation) of lymphocytes (16). The precise mechanism of its immunoregulatory effect, however, is completely unknown. For the experimentalist, glutathione may serve as a substitute for 2-mercaptoethanol for *in vivo* experiments. Application of 2-mercaptoethanol *in vivo* has previously been shown to augment the antibody response and to reconstitute in particular the reduced responsiveness of aged mice (33). The best effects were in this case, however, obtained if 2-

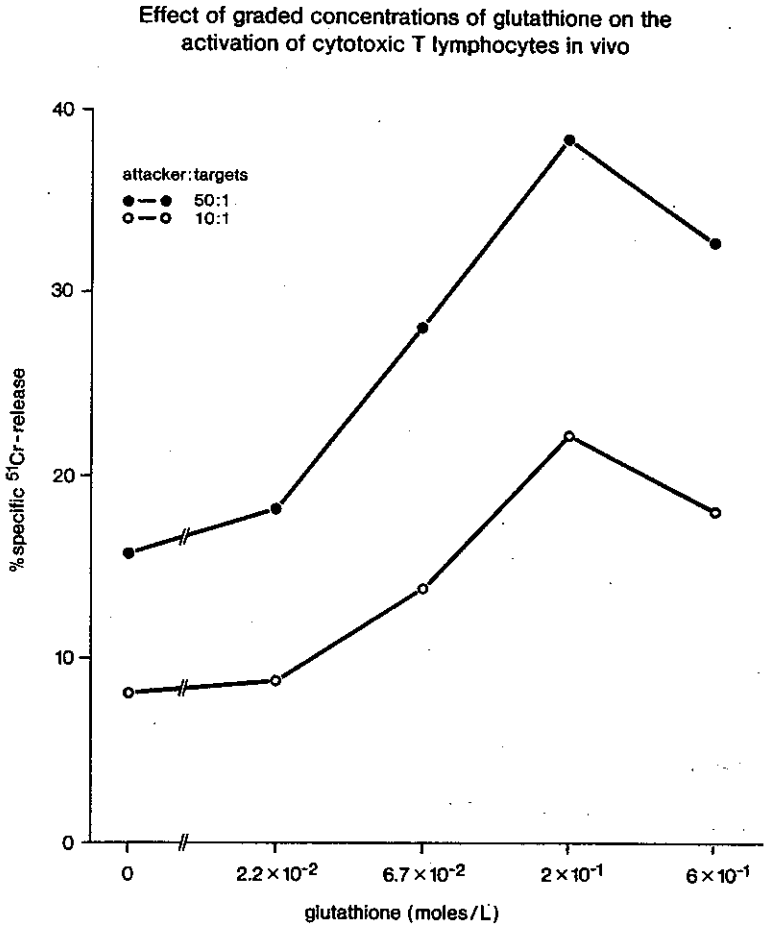

Fig. 2. Effect of graded doses of glutathione on the activation of cytotoxic T lymphocytes *in vivo*. The doses of 0.3 ml BSS containing the indicated concentrations of glutathione were injected into C3H Tif/Bom mice 2 and 3 days after immunization. For other details see Legend to Figure 1.

mercaptoethanol was administered just prior to or at the time of immunization. Whether glutathione can be used for the modulation of immune responses in clinical therapy remains to be investigated.

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