Biochemical Pharmacology, Vol. 33, No. 13, pp. 2061–2063, 1984.
Printed in Great Britain.

0006-2952/84 $3.00 + 0.00

THE EFFECT OF EMETINE ON THE IMMUNE RESPONSE OF MICE

ILDIKO CSUKA and FERENC ANTONI

1st Institute of Biochemistry, Semmelweis University Medical School, H-1444 Budapest P.O. Box 260, Hungary

(*Received* 25 *August* 1983; *accepted* 18 *January* 1984)

Abstract—Emetine (33 mg/kg body weight) administered intraperitoneally blocked the immune response of mice to 10^9 sheep red blood cells (SRBC). The inhibition was almost complete when the drug was administered simultaneously or 24 hr after immunization, while partial inhibition was caused by treatment at 48 and 72 hr. Incorporation of ^{14}C-leucine and ^{3}H-thymidine by spleen cells isolated 4 hr after emetine injection of the mice was strongly decreased. Incorporation was approaching the control level in cells isolated 72–96 hr after emetine administration. However, the incorporation of labeled precursors was less than after SRBC treatment only, even after 72–96 hr.

Emetine apparently blocked the development of immune response at an early stage and, in contrast to macromolecule synthesis, the inhibition of the antibody response was irreversible.

The antigens induce a series of functional and metabolic changes of various cells involved in the immune response. Disturbance of the cell interactions (between macrophages and T and B lymphocytes) and/or of the biosynthetic apparatus of the lymphocytes can block the antibody response at several sites.

In this study we describe the blocking effect of emetine on the immune response of mice. Emetine, a well-known inhibitor of protein synthesis [1–5] was administered to mice in order to investigate the disturbance of the induction of the synthesis of antibody to sheep red blood cells, and the impairment of macromolecule synthesis of spleen cells.

MATERIALS AND METHODS

Male CFLP mice, 8–10 weeks of age were used. Sheep red blood cells (SRBC) were stored in Alsever's solution for 1 week. For immunization SRBC was washed three times with isotonic saline then 10^9 cells were injected intraperitoneally in a volume of 0.1 ml phosphate buffered saline.

Subsequently (immediately, or after 24, 48 or 72 hr) emetine (generally 33 mg/kg in 0.1 ml PBS) was injected intraperitoneally with another syringe.

Spleen cells were isolated by gentle teasing of the organ with a pincette. The cells were washed three times with Hank's solution. The immune response against SRBC was quantitated by the lytic activity of serum or spleen cells.

The assay mixture contained 0.2 ml of diluted, heat-inactivated (at 56° for 30 min) serum or spleen cell suspension (containing 2×10^6 cells in Eagle's medium, if not stated otherwise), 0.2 ml of 15-fold diluted guinea pig complement absorbed with SRBC [16] and 0.2 ml of 1% suspension of SRBC containing 5×10^7 cells. The mixture was incubated at 37° for 60 min, then 2.4 ml isotonic saline was added. After centrifugation OD 413 nm of the supernatant was measured [16]. The background haemolysis in tubes containing SRBC only, or SRBC and spleen cells (or serum) resulted and OD 413 of 0.045. Protein and DNA synthesis was investigated by the incubation of 10^7 spleen cells in 1 ml Hank's solution for 1 hr at 37°, in the presence of 111 kBq ^{14}C-DL-leucine (469 MBq/mmole) or 111 kBq methyl-^{3}H-thymidine (742 GBq) mmole, both purchased from the Institute for Research, Production and Utilization of Radioisotopes, Prague). Incorporation was stopped by the addition of 1 ml 1 N perchloric acid. The resulting precipitate was washed three times with 0.5 N perchloric acid. Incorporation of radioactive leucine into protein was determined by dissolving the precipitate in 0.5 N NaOH and counting in a toluene based cocktail using a Beckman LS-355 liquid scintillation spectrometer. For the determination of the incorporation of ^{3}H-thymidine into DNA the perchloric acid precipitate was treated with 0.5 N perchloric acid at 90° for 30 min, then centrifuged. Radioactivity of the supernatant was counted in a toluene-based cocktail containing Triton X-100. Protein and DNA were measured as described [17, 18]. Incorporation was expressed as cpm/μg protein or DNA.

RESULTS

Intraperitoneal injection of SRBC to mice induced a marked and reproducible increase of 19S IgM lytic antibodies in the serum. High lytic titre of serum can be detected 96 hr after immunization, and spleen cells isolated at this time synthesize and secrete lytic antibodies *in vitro*. Simultaneous injection of various doses of emetine with SRBC resulted in a dose-dependent decrease of the production of lytic antibodies by spleen cells (Fig. 1).

Administration of 3.3 and 16.5 mg/kg body weight of emetine at the time of immunization inhibited the response of spleen cells by 20–30% while 33 mg/kg of emetine caused about 90% inhibition. Similarly, almost complete inhibition was achieved by two 16.5 mg/kg doses given at 0 and 24 hr. Importance

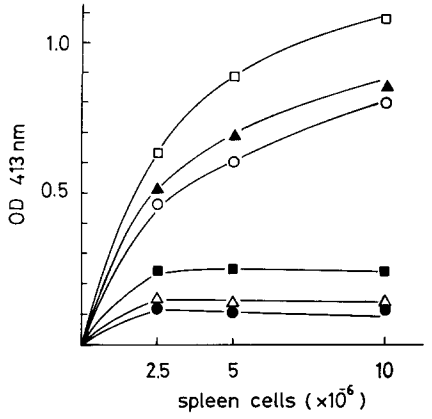

Fig. 1. Effect of different doses of emetine given at the time of immunization with SRBC on the lytic activity of spleen cells isolated after 96 hr. Haemolytic activity of antibodies produced by spleen cells was assayed as described in Materials and Methods. (●) Untreated control, (□) SRBC only. (▲) SRBC and 3.3 mg/kg emetine, (○) SRBC and 16.5 mg/kg emetine, (△) SRBC and 33 mg/kg emetine. In one case (■) 16.5 mg/kg emetine was given at the time of SRBC treatment, then 16.5 mg/kg was injected after 24 h. Each point represents the mean value of five samples.

of the timing of emetine administration is shown by the experiment presented in Fig. 2. Serum lytic titres increased steadily two days after the injection of SRBC. Emetine treatment (33 mg/kg) of mice at 0 or 24 hr completely abolished this response.

Emetine given at 48 hr prevented the further increase of the titre. At 72 hr this dose of the drug caused a small inhibition only. Similar results were obtained with spleen cells isolated 96 hr after immunization with SRBC (Fig. 3). Lytic antibodies were

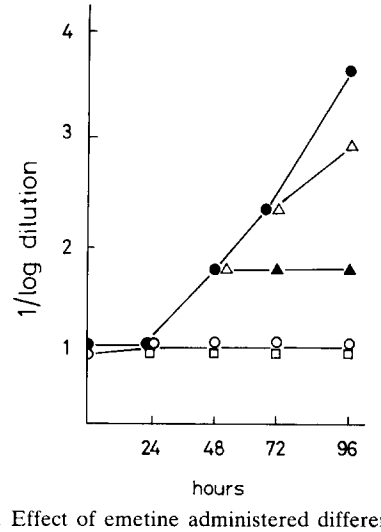

Fig. 2. Effect of emetine administered different intervals after immunization with SRBC on the lytic titre of the serum of mice. Haemolytic assay was performed as described in Materials and Methods. Controls received SRBC only (●), the other animals received SRBC and 33 mg/kg emetine. Emetine was given at 0 hr (□), 24 hr (○), 48 hr (▲) or 72 hr (△). Each point represents the mean value of five samples.

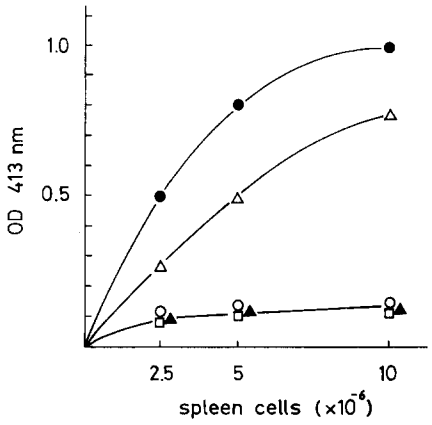

Fig. 3. Effect of emetine given at different intervals after immunization with SRBC on the lytic activity of spleen cells isolated after 96 hr. Experimental conditions are described in Material and Methods. Controls received SRBC only (○). The rest of animals received SRBC and 33 mg/kg emetine. Emetine was given at 0 hr (□), 24 hr (○), 48 hr (▲), 72 hr (△). Each point represents the mean value of five samples.

not produced by cells isolated from animals which were treated with emetine at 0, 24 or 48 hr after immunization. However, the inhibition was only 30% in the case of cells from animals which received emetine at 72 hr.

In order to relate the inhibition of the synthesis of specific antibodies to total protein synthesis, we investigated the *in vitro* incorporation of ^{14}C-leucine into the spleen cells of animals which were treated *in vivo* with SRBC and/or emetine, then sacrificed 4, 24, 48, 72 and 96 hr after the treatment (Fig. 4A). Immunization with SRBC caused a marked increase of incorporation culminating at 72 hr. Emetine inhibited protein synthesis by about 60% at 4 hr already, but the rate of incorporation returned to the control level after 96 hr. No marked difference was seen between cells of immunized and non-immunized mice in this respect. It is noted that in spite of apparently normal protein synthesis after 96 hr, lytic antibodies could not be demonstrated 96 hr after the simultaneous injection of SRBC and emetine.

DNA synthesis of spleen cells was investigated under similar circumstances (Fig. 4B). *In vivo* SRBC treatment caused an increase of ^{3}H-thymidine incorporation into DNA of spleen cells isolated at various intervals. The inhibitory effect of *in vivo* emetine treatment (with or without immunization) lasted for about 48 hr, then the rate of incorporation gradually returned to control level in three days. However, at the time of the peak of SRBC induced stimulation of DNA synthesis (72 hr) the rate of (emetine inhibited) incorporation was higher in the cells of immunized animals as compared to that of the spleen cells of non-immunized, emetine treated mice.

DISCUSSION

Emetine inhibits protein synthesis in eucaryotes by interfering with ribosome translocation along mRNA [14]. An alteration of the ribosomal protein

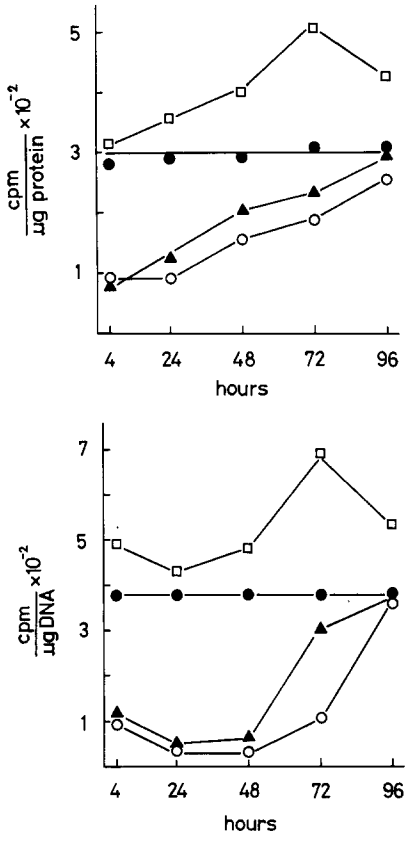

Fig. 4(A). Effect of SRBC and emetine treatment of mice on the incorporation of ^{14}C-leucine into protein of isolated spleen cells. The animals were sacrificed at the indicated intervals after the immunization. Experimental conditions are described in Materials and Methods. (●) Untreated control, (○) 33 mg/kg emetine, (□) SRBC, (▲) SRBC and emetine 33 mg/kg. Each point represents the mean value of five samples. (B) Effect of SRBC and emetine treatment of mice on the incorporation of ^{3}H-thymidine into DNA of isolated spleen cells. The animals were sacrificed at the indicated intervals after the immunization. Experimental conditions are described in Materials and Methods. (●) Untreated control, (○) 33 mg/kg emetine, (□) SRBC, (▲) SRBC and emetine 33 mg/kg. Each point represents the mean value of five samples.

S14 has resulted in emetine resistance in a mutant line of chinese hamster ovary cells [15]. Emetine also inhibits DNA synthesis [1, 13]. We have previously demonstrated its inhibitory effect on RNA synthesis in lymphocytes [6].

What is the consequence of the inhibition of macromolecule synthesis by emetine during the *in vivo* development of a specific immune response? The results presented in this paper demonstrated the grave disturbance of immune reactivity, as shown by the lack of serum antibodies against SRBC after simultaneous treatment with emetine and SRBC. Spleen cells of mice treated in this manner also failed to produce lytic antibodies in an *in vitro* test system. We obtained similar results when emetine was injected 24–48 hr after immunization (Figs. 1–3). Emetine administered 72 hr after immunization had a moderate inhibitory effect only [19]. It has been reported that cyclophosphamide exerts a similar inhibition on the immune response to SRBC [20].

We concluded that the early events of the immune response were sensitive towards emetine. This can be explained by the prompt *in vivo* inhibition of protein and DNA synthesis in spleen cells, which persisted *in vitro* (Fig. 4).

Emetine has been shown to be retained by the organism for a long time; in mice 35% of emetine administered i.p. still persists 8 days later [7, 10]. However, protein and DNA synthesis were relieved from inhibition in our experiments after 96 hr. Nevertheless, no specific antibodies were detected after this interval either. Though the inhibition of macromolecule synthesis was reversible, the development of the immune response was irreversibly blocked at some early stage. The induction of antibody synthesis requires complex cooperation of several different types of cells. Our preliminary experiments suggest that the primary effect of emetine is the disturbance of T cell functions, since the T cells lost their E rosette forming capacity. This may explain the failure to respond to SRBC a well known T cell dependent antigen.

Acknowledgements—We appreciate the skilful technical assistance of Mrs Ilona Mongyi and Mrs Agnes Temesi. We thank Dr. Károly Mészáros for reading the manuscript and Mrs Aniko Varga for expert secretarial assistance. This work was supported by the Ministry of Health of Hungary.

REFERENCES

1. A. P. Grollman, *J. biol. Chem.* **243**, 4089 (1968).
2. A. P. Grollman, *Proc. natn. Acad. Sci.* **56**, 1867 (1966).
3. A. P. Grollman and M. T. Huang, *Fedn. Proc.* **32**, 1673 (1973).
4. A. P. Grollman and Z. Jarkovsky, in *Antibiotics III. Mechanism of Action* (Corcoran and Hahn, Ed.) Springer (1975).
5. N. Entner and A. P. Grollman, *J. Protozol.* **20**, 160 (1973).
6. Gy. Farkas, F. Antoni, M. Staub and P. Piffkó, *Acta Biochem. Biophsy. Acad. Sci. Hung.* **9**, 63 (1974).
7. A. I. Gimble, C. Davison and P. K. Smith, *Pharm. Exp. Therap.* **94**, 931 (1948).
8. Ch. I. Flickinger, *Exptl Cell. Res.* **74**, 541 (1972).
9. N. L. Oleinick, *Archs. Biochem. Biophys.* **182**, 171 (1977).
10. W. C. T. Yang and M. Dubick, *Pharmac. Ther.* **10**, 15 (1980).
11. R. K. Johnson and W. R. Jondorf, *Biochem. J.* **126**, 22 (1972).
12. R. K. Johnson and W. R. Jondorf, *Biochem. J.* **140**, 87 (1974).
13. R. S. Gupta and L. Siminovitch, *Cell* **9**, 213 (1976).
14. R. S. Gupta and L. Siminovitch, *Biochemistry* **16**, 3209 (1977).
15. J. J. Madjar, K. Nielsen-Smith, M. Frahm and D. J. Roufa. *Proc. natn. Acad. Sci. U.S.A.* **79**, 1003 (1982).
16. M. A. Simpson and J. J. Gozzo, *J. Immunol. Meth.* **21**, 159 (1978).
17. K. Burton, in *Methods in Enzymology* (Ed.: L. Grossman and Moldave) XII., Part B. 163 (1968).
18. O. H. Lowry, N. J. Rosenbrough, A. L. Farr and R. J. Randall, *J. Biochem.* **193**, 265 (1951).
19. I. Csuka, Á. Soóki-Tóth and K. Mészáros, *Acta Biochem. Biophys. Acad. Sci. Hung.* **17**, 65 (1982).
20. M. Liske, *J. Immunol. Meth.* **34**, 225 (1980).