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ENHANCEMENT BY α-1-ANTICHYMOTRYPSIN OF ANTIBODY RESPONSE *in vivo*

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Summary: The immunological effect of α-1-antichymotrypsin on the antibody response *in vivo* was studied in mice. The plaque-forming cell response to sheep erythrocytes was enhanced by an intravenous injection of α-1-antichymotrypsin. α-1-antichymotrypsin was effective if injected 2 days before or simultaneously with the injection of sheep erythrocytes. Immunoadsorption with an anti α-1-antichymotrypsin-Sepharose column indicated that α-1-antichymotrypsin itself was responsible for enhancement of plaque-forming cell response.

A serum DNA-binding protein with molecular weight of 64,000(64DP) was described by Katsunuma et al(1). 64DP was shown to be α-1-Achy previously by Siddiqui et al(2). At the fourth international symposium on the prevention and detection of cancer(Aug, 1980, London), we reported that purified 64DP was identified as α-1-Achy by the Ouchterlony double diffusion method and immuno-electrophoresis using anti α-1-Achy antiserum(Hoechst, Japan) and anti 64DP antiserum. There is a strong similarity of molecular weight and amino acid composition between 64DP and α-1-Achy(kindly supplied from Behring institute, West Germany).

The concentration of this protein is 180-260 μg/ml in normal serum and increases in serum of inflammatory states or malignant diseases(3). It is known to be one of acute phase reactant proteins. Some acute phase reactants serve as suppressors or enhancing substances of immune response. α-1-antitrypsin and immunoregulatory α-globulin suppress the *in vivo* and *in vitro* antibody responses of mouse spleen cells against SRBC(4)(5). α-1-acid glycoprotein also suppresses the proliferative response to mitogens and allogenic cells(6)(7) and antibody response to SRBC(7). On the other hand transferrin enhances the proliferative

Abbreviations: α-1-Achy, α-1-antichymotrypsin; SRBC, sheep erythrocytes; PFC, plaque-forming cell.

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response of human lymphocytes to mitogens as measured by morphologic transformation and [^{3}H]thymidine incorporation(8).

α-1-Achy has been reported to inhibit some proteases such as pancreatic chymotrypsin, leukocyte cathepsin G or mast cell chymotrypsin and not to inhibit pancreatic trypsin and leukocyte elastase(9). However, little is known about its effect on the immune response. The data presented here indicate that α-1-Achy has an immunoenhancing effect on the in vivo PFC response to SRBC in mice.

MATERIALS AND METHODS

Animals. Specific pathogen-free BALB/c male mice were obtained from Charles River(Japan). The mice employed were 8-11 weeks old(26-29 g).

Antigen. SRBC were obtained from Nippon Bio-Test Laboratories(Japan) and stored in Alsever's solution at 4°C. Before use, the SRBC were washed three times with sterile physiological saline and suspended in saline at 10%. A suspension containing $4x10^8$ SRBC (0.2 ml) was intravenously injected into mice.

Preparation of α-1-Achy. The purification of α-1-Achy was performed with the method of purification procedure of 64DP described previously(1). The homogeniety of this protein was established by sodium dodecyl sulfate(SDS)-polyacrylamide gel electrophoresis(10) and normal polyacrylamide gel electrophoresis(11). The final purified protein was judged to be α-1-Achy by the Ouchterlony double diffusion method(12) using commercial anti α-1-Achy antibody (Hoechst, Japan) and anti 64DP antibody made by us.

Affinity column chromatography of α-1-Achy. Rabbit antiserum against human α-1-Achy was prepared by an injection of the purified α-1-Achy in Freund's adjuvant. The antiserum was judged to be specific by the Ouchterlony double diffusion method and immunoelectrophoresis. Anti α-1-Achy IgG isolated from antisera against α-1-Achy was coupled to CNBr-activated Sepharose 4B. The affinity column was pre-eluted with 3M NaSCN in physiological saline, before application of the sample. The sample containing 10 mg of purified α-1-Achy in 1.6 ml physiological saline was applied to the column. After washing with physiological saline, the column was eluted with 3M NaSCN in saline. The non-adsorbed fractions and the adsorbed fractions were collected and concentrated to 2.0 ml and 1.0 ml, respectively, by Collodion-bag(Sartorius Membranfilter GmbH, West Germany). After dialysis against physiological saline, they were stored at 4°C. The concentration of α-1-Achy in the adsorbed fraction was 3.12 mg/ml and protein in the non-adsorbed fraction was not detected by the Lowry method(13).

Antibody forming cell assay. The mice which were injected with SRBC, or with SRBC and α-1-Achy were sacrificed by cervical dislocation 5 days after immunization with SRBC. Their spleens were individually teased in Eagle's minimum essential medium(MEM) and passed through a stainless steel screen to obtain a single cell suspension. The number of PFC in the spleen was determined by the Jerne hemolytic plaque assay(14).

RESULTS AND DISCUSSION

The immunological effect of α-1-Achy on anti-SRBC PFC response in vivo was studied by injecting various doses of α-1-Achy into groups of BALB/c mice. 0.5-2.0 mg of α-1-Achy was intravenously injected into mice together with $4x10^8$

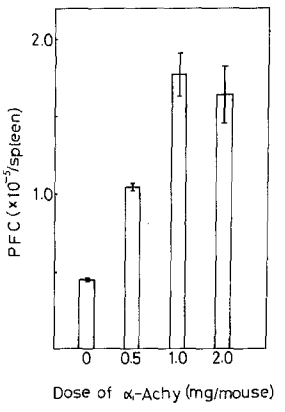
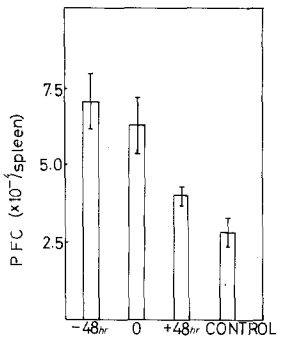


Fig. 1. The effect of α-1-Achy on the anti-SRBC PFC response in mice. Each bar represents the average number(±S.E.) of PFC with five mice. The number of direct PFC was measured at 5 days post-immunization.

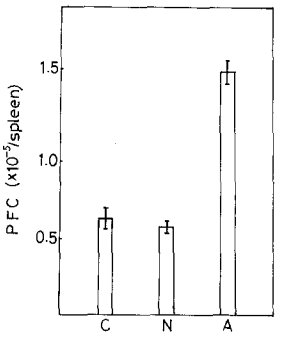
SRBC. The results presented in Fig.1 indicate that these doses of α-1-Achy significantly enhance the anti-SRBC PFC response. α-1-Achy was capable of enhancing the PFC response by 230, 390, and 370% after an injection of 0.5, 1.0, and 2.0 mg of α-1-Achy respectively. The optimal dose of α-1-Achy was 1 mg per mouse. At 10 minutes, 2 hours, 6 hours, and 16 hours after an injection of 1 mg of α-1-Achy into mice, concentrations of α-1-Achy in serum of mice were 1230, 464, 290, and 93 μg/ml. After 4 days, α-1-Achy was not detected in serum of mice. The level of α-1-Achy in serum after 10 minutes of injection is higher than the normal human level(180-260 μg/ml), but the same as the level of α-1-Achy in serum with some malignant diseases, such as malignant ovarian tumor or progressive gastric cancer.

Without the administration of SRBC antigen, the injection of 1 mg of α-1-Achy nonspecifically increased the number of PFC against xenogenic erythrocytes in the mouse spleen to twice the control level. The number of PFC of mice without α-1-Achy was 112±9.8 per spleen and of mice with α-1-Achy was 264±60 per spleen (mean±S.E.).

An immunoeffective dose of α-1-Achy(1 mg/mouse) was injected into mice at 48 hours before, simultaneously with, or 48 hours after the injection of 4×10^8 SRBC antigen. We found that α-1-Achy was effective if injected 48 hours before



(2)



(3)

Fig. 2. The temporal relationship between administration of α-1-Achy and SRBC on PFC response. Each bar represents the average number(±S.E.) of anti-SRBC PFC with five mice. SRBC alone was injected at day 0 as a control. The number of PFC was measured at 5 days post-immunization.

Fig. 3. Immunoadsorption atudy. Each bar represents the average number(±S.E.) of anti-SRBC PFC with five mice except for the adsorbed fraction which represents the average number(±S.E.) of PFC with three mice. SRBC alone was injected as a control. The dose of α-1-Achy per mouse was less than 8×10^{-4}mg in the non-adsorbed fraction and 1 mg in the adsorbed fraction. The number of PFC was measured at day 5.
C: control. N: non-adsorbed fraction. A: adsorbed fraction.

or simultaneously with antigen. After 48 hours, α-1-Achy enhanced anti-SRBC PFC response by 141%(Fig.2).

In order to examine whether other substances which enhance the PFC response were present in α-1-Achy preparation, it was adsorbed on an anti α-1-Achy antibody-Sepharose affinity column(Fig.3). 0.2 ml of the non-adsorbed fraction (containing less than 8×10^{-4} mg of α-1-Achy) or 0.32 ml of the adsorbed fraction(containing 1 mg of α-1-Achy) was injected simultaneously with SRBC into mice. The adsorbed fraction enhanced PFC response to SRBC to 2.6 times the control level, whereas the non-adsorbed fraction did not enhance. This result indicates that α-1-Achy itself enhances PFC response to SRBC.

There is evidence that many serum proteins have immunosuppressive effects. Few, however, enhance immune response. We have found that α-1-Achy enhances immune response in vivo. Studies concerning the mechanism of action of α-1-Achy on antibody response are now in progress; the immunological role of α-1-Achy in malignant diseases in which its serum level increases remains unknown.

REFERENCES

1. Katsunuma,T.,Tsuda,M.,Kusumi,T.,Ohkubo,T.,Mitomi,T.,Nakasaki,H.,Tajima,T., Yokoyama,S.,Kamiguchi,H.,Kobayashi,K., and Shinoda,H.(1980) Biochem.Biophys Res.Commun.93,552-557.
2. Siddiqui,A.A.,Hughes,A.E.,Davies,R.J.H., and Hill,J.A.(1980) Biochem. Biophys.Res.Commun.95,1937-1742.
3. Nakasaki,H.,Mitomi,T.,Tajima,T.,Ogoshi,K.,Tsuda,M., and Katsunuma,T.(1981) Gann. in press.
4. Arora,P.K.,Miller,H.C., and Aronson,L.D.(1978) Nature(London).274,589-590.
5. Badger,A.M.,Merluzzi,V.J.,Mannich,J.A., and Cooperband,S.R.(1977) J.Immunol 118,1228-1231.
6. Chiu,K.M.Mortensen,R.F.,Osmand,A.P., and Gewurz,H.(1977) Immunology.32, 997-1005.
7. Bennet,M., and Schmid,K.(1980) Proc.Natl.Acad.Sci.77,6109-6113.
8. Tormey,D.C., and Mueller,G.C.(1972) Exptl.Cell.Res.74,220-226.
9. Travis,J.,Bowen,J., and Baugh,R.(1978) Biochmistry.17,5651-5656.
10. Laemmli,U.K.(1970) Nature(London) 227,680-685.
11. Davis,B.J.(1964) Ann.N.Y.Acad.Sci.121,404-427.
12. Ouchterlony,O.(1958) in Progress in Allergy. vol 5, page 1-78, Karger, New York.
13. Lowry,O.H.,Rosenbrough,N.J.,Farr,A.L., and Randall,R.J.(1951) J.Biol.Chem. 193,265-275.
14. Jerne,N.K.,Nordin,A.A., and Henry,C.(1963) in Cell bound antibodies. page 109-116,Wister Institute Press, Philadelphia.