EFFECT OF SELECTED AMINO ACID AND AMINO SUGAR DERIVATIVES ON THE IMMUNE RESPONSE[1]

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Abstract. The low molecular weight amino acids, N-α-benzyloxycarbonyl-L-lysine and L-lysine, and the amino sugar derivative, N-acetyl-D-glucosamine, were examined for effects on the immune response of mice to sheep erythrocytes. Treatment dosages of 1000, 100, 50 and 10 mg/kg of the test compounds did not alter the body weight, organ weight or peripheral leucocyte count of test mice. Mice given N-α-benzyloxycarbonyl-L-lysine demonstrated significant increases or decreases in the splenic plaque-forming cell response to sheep erythrocytes depending on the treatment regimen employed. Animals given N-acetyl-D-glucosamine showed an increase in the splenic plaque forming cell response while those receiving L-lysine had no detectable alteration.

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Considerable experimentation has been devoted to the selective control of the immune response. Among these studies are the findings that the immunoglobulin molecule may be influential in immune regulation (Uhr and Moller 1968, Bystryn *et al* 1971, Watson 1973). The research in our laboratories is concerned with the synthesis and analysis of synthetic analogues of IgG and their influence on the immune system. These compounds are based on the structural features of human IgG (Edleman *et al* 1969) and the sugar moiety N-acetyl-D-glucosamine (NAG) common to many glycoproteins (Spiro 1970). Prior to analysis of the synthetic analogues, it was necessary to establish the possible alterations in the immune response which occur due to agents representative of chemical precursors and metabolites of the synthetic derivatives. The present investigation was undertaken to examine the influence of the sugar moiety NAG, the synthetic amino acid derivative N-α-benzloxycarbonyl-L-lysine (Cbz-Lys), the naturally-occurring amino acid L-lysine (Lys), and combinations of these compounds on the splenic plaque forming (PFC) response of mice to sheep erythrocytes.

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MATERIALS AND METHODS

Mature female C3H/HeJ mice (20–25 g) from Jackson Laboratories, Bar Harbor, ME were housed in plastic cages (4/cage) and allowed free access to rodent chow (Purina) and water through the treatment period. The challenge antigen employed was the sheep erythrocyte, collected in Alsever's solution, and obtained from donor sheep housed in our animal facility. Sheep erythrocytes were washed three times in 0.15M NaCl prior to the intraperitoneal injection of approximately 10^8 cells per mouse.

The Cbz-Lys/NAG was administered as a 50:50 mixture, by weight. In addition, Lys NAG, and Cbz-Lys were evaluated. The test compounds were administered intraperitoneally at 24 hr intervals to groups of antigen-pretreated animals. Control groups received a similar regimen of Hanks' balanced salt solution (GIBCO, Grand Island, NY). Mice in both control and treated groups were weighed at the initiation and again at the terminiation of each experiment and spleen and thymus weights were determined at the time of sacrifice.

Plaque-forming cell numbers were determined by a modification of the Jerne and Nordin plaque assay (Jerne and Nordin 1963). This measured the effect of drug treatment on the number of PFC/10^6 splenic cells in the primary immune response. Routinely, the spleens of all six mice were pooled for the cell suspension and the results of six plates per experiment were expressed as plaque-forming cells (PFC) per 10^6 spleen cells assayed. IgG plaques were developed by adding 0.2 ml of a 1:50 dilution of

rabbit anti-mouse IgG serum (Miles Laboratories, Elkhart, IN). IgM responses were determined 4 days following challenge with Sheep red blood cells and IgG responses 5 days following antigen challenge. Preliminary testing in control C3H/HeJ mice demonstrated that the maximum IgM and IgG responses were observed at these intervals.

Percentage thymus-dependent (T) lymphocytes present in the spleens and thymuses of animals was determined by a direct cytotoxicity test employing rabbit anti-mouse brain serum prepared according to the method of Golub (1971). In addition, the mitogenic response of T cells to Con A (Nutritional Biochemicals, Cleveland, OH) and phytohemagglutinin (PHA; Burroughs-Wellcome, Tuckahoe, NY) and B cells to lipopolysaccharide (DIFCO Laboratory, Detroit, MI) were assessed employing the method of Adler and coworkers (1970).

RESULTS

Treatment dosages of 1000, 100, 50 and 10 mg/kg of the test compounds administered for periods as long as three days did not appear to alter the body weight, organ weight or peripheral leucocyte counts of test mice. Experiments employing equimolar mixtures of Cbz-Lys/NAG at the two lower treatment levels of 50 or 10 mg/kg indicated that either enhancement or depression of the splenic PFC response could be observed depending on the assay time and treatment interval relative to administration of the sheep red blood cells. A significant increase in the IgM splenic PFC response was observed in mice given a single injection of either the 10 or 50 mg/kg dosage of Cbz-Lys at similar dosages before assay on day 4 (table 1). Conversely, a significant decrease in the IgG response (table 2) was noted in mice given 50 or 10 mg/kg dosages daily for 2 days prior to assay on day 5 of Cbz-Lys/NAG or Cbz-Lys. However, the response of mice given NAG only in this treatment regimen was elevated. An evaluation of other selected injection regimens of these agents did not elicit changes in the splenic PFC response. Administration of L-lysine produced no detectable alterations regardless of the time intervals or dosages examined.

It is not clear if the Cbz-Lys/NAG mixture was responsible for the increase in the splenic PFC response or if the effect was due to the NAG alone. Further studies will be necessary to evaluate

TABLE 1

*Effect of N-acetyl-D-glucosamine, N-α-benzyloxycarbonyl-L-lysine, (CBz-Lys) and N-α-benzyloxycarbonyl-L-lysine/N-acetyl-D-glucosamine on IgM splenic PFC response to SRBC in C3H/HeJ mice.**

Treatment Dosages**	IgM producing PFC/10^6 spleen cells±SEM***	
	Trial 1	Trial 2
50 mg/kg Cbz-Lys/NAG	475±12	382±19
10 mg/kg Cbz-Lys/NAG	627±55	512±27
HBSS (Controls)	372±17	289±31
50 mg/kg NAG	572±39	471±16
10 mg/kg NAG	501±62	432±27
HBSS (Controls)	302±31	254±18
50 mg/kg Cbz-Lys	160±21	124±20
10 mg/kg Cbz-Lys	185±41	138±32
HBSS (Controls)	312±27	271±23

*Each study represents 6 replicate assays conducted using 4 animals per assay for each separate trial. Cbz-Lys/NAG mixtures were 1:1 by weight.

**Treatment was administered 24 hours prior to assay. Assay for splenic IgM producing cells was conducted 4 days after challenge with sheep red cells.

***All test values were significantly different from controls ($P \leq 0.05$).

TABLE 2

*Effect of N-acetyl-D-glucosamine. N-α-benzyloxylysine/N-acetyl-D-glucosamine on IgG splenic PFC response to SRBC in C3H/HeJ mice.**

Treatment Dosages**	IgG producing PFC/10^6 spleen cells±SEM***	
	Trial 1	Trial 2
50 mg/kg Cbz-Lys/NAG	110±47	89±21
10 mg/kg Cbz-Lys/NAG	153±19	112±14
HBSS (Controls)	347±69	279±19
50 mg/kg NAG	385±71	327±24
10 mg/kg NAG	309±24	298±14
HBSS (Controls)	213±10	218±23
50 mg/kg Cbz-Lys	37± 8	42± 9
10 mg/kg Cbz-Lys	49±12	53±16
HBSS (Controls)	395±52	298±31

*All studies represent 6 replicate assays conducted using pooled spleens from 4 animals per assy for each separate trial. Cbz-Lys/NAG mixtures were 1:1 by weight.

**Test dosages were administered 48 and 24 hrs. prior to assay. Assay for splenic IgG producing cells was conducted 5 days after challenge with sheep red cells.

***All test values were significantly different from controls ($P \leq 0.05$).

this possibility since similar trends were observed in Trial 1 and Trial 2 for both the IgM and IgG responses.

The observed changes in the splenic PFC response do not appear to be the result of selective toxic activity towards the T or B lymphocytes. An increase similar to control groups in the percentages of splenic T cells was noted for mice given either Cbz-Lys/NAG (50:50) or Cbz-Lys at any treatment interval or dosage. Tests in unchallenged mice also indicated the compounds elicited no measurable change in T cell numbers. In addition, the mitogenic response of T cells to Conconavalen A and phytohemagglutinin and the response of B cells to lipopolysaccharide was comparable in both control and treatment groups.

DISCUSSION

It has been shown that the intracellular synthesis of the protein chain in a plasma tumor which secretes a K light chain can be disrupted by methylthioinosine periodate, by virtue of its ability to block intracellular attachment of hexoses (Cronenberger and Kimball 1971). The capability of small peptides to inhibit protein synthesis has been demonstrated in the case of peptide antibiotics (Garrod and O'Grady 1968). The influence of the test compounds used was observed during the latter stages of the immune response when immunoglobulin production and secretion were occurring. This suggests that the effects observed may be due to some modification of the production and/or secretion of immunoglobulins.

Another possiblity to be considered is that the compounds evaluated in the present study may have functioned as modulator molecules. Compounds such as teichoic acid, lipopolysaccharide, lectins and gamma globulins have been shown to enhance or suppress the immune response in mice depending on the dosage and time interval of administration (Schwab 1975). Although the compounds examined in the present study were not as complex as those previously shown to act as modulator molecules, the specific active portion of these materials is not well defined. Thus, it may be that our test compounds were capable of assuming a similar role and the observed effects reflected this activity.

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