PREPARATION OF TWO SOLID PHASE IMMUNOGENS

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Summary

Dinitrophenyl (DNP) conjugates of Bio-Gel and dinitrophenyl-ornithine conjugates of Bio-Gel and Sepharose were prepared. Their efficacy as immunogens was assessed. It was found that the dinitrophenyl-ornithine gels ellicited generation of large numbers of splenic anti-DNP plaque-forming cells. DNP-Bio-Gel was not immunogenic.

Chemical modification of agarose (1) and polyacrylamide (2) beads has allowed preparation of a variety of derivatives. Such derivatives have been used as carriers for small and large biochemically active molecules. A wide range of applications in affinity chromatography have been utilized, including purification of immunochemicals on antigen- or antibody-charged columns of Sepharose and Bio-Gel (3). Recently, affinity chromatography has been applied to the selective adsorption and isolation of immune cell populations (4). Specific binding of activated cells to antigen matrices suggested their application as immunizing agents for induction of immune events. Such conjugates have not been used previously in methods of immunization, and preparations of immunogenic, haptenated Sepharose and Bio-Gel are described.

MATERIALS AND METHODS

Preparation of dinitrophenyl-bearing Sepharose

Sepharose 2-B (Pharmacia Fine Chemicals, Upsalla) was washed three times by suspension and centrifugation in eight volumes water to remove sodium azide preservative. Ten ml washed, packed Sepharose was brought to 20 ml in 0.1 N sodium bicarbonate and activated with cyanogen bromide as described by Cautrecasas (1), but at room temperature. Following activation, the gel was washed in 400 ml volumes of bicarbonate and resuspended to 10 ml. Thirteen mg delta-N-2,4 dinitrophenyl-L-ornithine-HCL (Sigma Chem. Co., St. Louis), dissolved in 3.5 ml bicarbonate buffer and adjusted to pH 9 was added and stirred gently at 4°C for 24 hours. 2,4-Dinitrophenyl-Ornithine-Sepharose (DNP-O-Seph) was washed extensively in phosphate buffered saline and stored in presence of 0.1% sodium azide.

Preparation of dinitrophenyl-bearing Bio-Gel

Twenty-five ml Bio-Gel P-6 (50-100 mesh, Bio-Rad Laboratories, Richmond, Ca) were hydrated and converted to the hydrazide derivative (2). (a) 2.7 g recrystallized 2,4-dinitrobenzenesulfonate dissolved in 75 ml 0.09 M $Na_2B_4O_7$ was added to 25 ml settled hydrazide and stirred overnight at room temperature. The dinitrophenylated beads (DNP-Bio-Gel) were washed extensively in 0.2M NaCl - 0.2M Tris, pH 8.5, water and then suspended in phosphate buffered saline with sodium azide. (b) 25 g of the hydrazide were converted to the acylazide derivative (2). Two hundred mg dinitrophenyl-ornithine, in 0.2 M Na_2CO_3, adjusted to pH 9.4 was added to 10 ml of the settled derivative, stirred for 2 hours at 0°C, and finally washed in water. Unreacted groups were quenched in 500 ml - 2M NH_4Cl - 1M NH_4OH, pH 9.1 overnight. These conjugated beads (DNP-O-Bio-Gel) were also extensively washed and stored as described previously.

Immunization

Young Balb/c female mice were injected intraperitoneally through 16 gauge needles with 1 ml haptenated gels at the following injectable dilutions: 3 parts DNP-O-Seph : 1 part saline; 1 part DNP-O-Bio-Gel : 1 part NaCl; and 1 part DNP-Bio-Gel : 1 part NaCl.

Assessment of anti-DNP immunity

The Mischell-Dutton modification (5) of the Jerne plaque assay (6) utilizing dinitrophenylated sheep erythrocytes prepared with dinitrofluorobenzene (7) was used to assess numbers of splenic anti-DNP plaque-forming cells (PFC).

RESULTS AND DISCUSSION

Hapten content of gels

Spectrophotometric analysis of supernatant fluids at 360 nanometers was used to evaluate uptake by gels of DNP and DNP-Ornithine. Bio-Gel was found to possess 17.5 mg DNP and 9.5 mg DNP-Ornithine per ml of settled gel, while Sepharose was found to possess 0.78 mg per ml.

Plaque forming cell responses

Injection of DNP-O-Seph was followed by substantial increases of DNP-PFC (Table 1). Generally, peak numbers of direct PFCs were found on day 5. Mice immunized with

Immunizing Conjugate	Direct plaque forming cells/spleen on day: 1	3	5	7
DNP-Ornithine-Sepharose	1000 ± 500*	9170 ± 519	17190 ± 1030	12730 ± 1153
DNP-Ornithine-Bio-Gel	1525 ± 387	29925 ± 1062	20395 ± 2883	6000 ± 1500

Table 1. Direct splenic plaque forming cells found as a result of intraperitoneal injection of dinitrophenyl-ornithine-Sepharose or dinitrophenyl-ornithine-Bio-Gel. At least 4 mice each were sacrificed 1, 3, 5, and 7 days following immunization. Spleen cells were subjected to a modified Jerne plaque assay. Means of anti-DNP PFC are followed by values for standard errors of the means (*).

DNP-O-Bio-Gel also exhibited increased numbers of anti-DNP-PFCs (Table 1). Maximum numbers were reached by 3 days. Thereafter splenic antibody forming cells declined with great rapidity. Interestingly DNP-Bio-Gel was not immunogenic.

Hapten conjugated gels have been utilized as immunoadsorbents in cell fractionation procedures. Since they specifically bind antigen-reactive as well as antibody forming cells in affinity chromatography (8) it was logical to attempt their use as inductional agents as well. Numbers of anti-hapten plaque forming cells found in spleens of animals immunized with these solid phase immunogens represent a substantial response in comparison to those induced by complex immunogenic determinants (6).

Studies of anti-hapten binding-constants indicate that antibody reacts not only with hapten but with its amino acid ligand as well (9). From results presented above, immunogenicity of DNP appears to depend on the presence of Ornithine between the hapten and bead surface. Such a consequence suggests that the derivatized gels themselves do not contribute to immunogenicity of the hapten.

Haptenated solid phase immunogens may provide opportunity to study phenomena of induction of immunogenesis. Sepharose and Bio-Gel are extremely stable under physiological conditions and it is not likely that these solid phase immunogens are metabolically destroyed. This attribute, along with the fact that they may participate only as vehicles for the haptenic determinant, make them of interest to in-depth studies of immune phenomena.

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