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Environmental Galactans Which React With IgA Myeloma T191B[1] (38809)

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Six different Balb/c IgA myeloma proteins have been found which bind multiple β-D (1 → 6)-linked β-galactopyranose residues. They are J1, S10, X24, X44, J539, and T191B (1). The independent production of different myeloma proteins with the same activity suggests that common natural antigens are present in the environment of the Balb/c mouse and that plasmacytomas may have their origin from precursor cells responding to these natural antigens (2). In fact, it has shown been that S10 and T191B myeloma proteins precipitate with antigenic materials present in food and bedding of mice (3). We now report additional galactans present in the environment of the mouse which precipitate with T191B. One of them, a phosphogalactan from Sporobolomyces sp. consists of approximately equal amounts of α1, 6- and α1, 3-galactosyl linkages (4).

Materials and Methods. Myeloma tumor T191B was obtained from Dr. Michael Potter (National Cancer Institute, Bethesda, MD) and was maintained in Balb/c female mice by subcutaneous passage of tumor fragments. Animals were bled from the ophthalmic plexus after the appearance of a palpable mass and the sera from these animals were pooled.

Quantitative microprecipitin reactions were carried out by adding various concentrations of polysaccharide antigens to 70-μl aliquots of T191B serum and incubating the reactants for 24 hr at 4°C. The *N* content of the precipitates which formed was determined by Nesslerization (5). Inhibition assays were performed by mixing the inhibiting hapten with T191B serum before the addition of an amount of polysaccharide antigen which gave maximal precipitation.

Screening tests for pollen extracts which would precipitate with T191B serum were carried out by adding 0.025 ml of extract to 0.025 ml of serum, adjusting the volume to 2 ml with isotonic saline, and incubating the mixture at 37°C for 15 min and then overnight at 4°C. In most cases precipitation occurred within minutes. Crude pollen extracts were prepared by extracting defatted pollen grains (Greer Laboratories) overnight at 4°C with Coca's solution (20% w/v). An arabinogalactan from Timothy pollen was isolated from a crude Timothy pollen extract by serial alcohol precipitation followed by chloroform treatment to remove protein (6). Further purification was carried out using Biogel P-200 columns, and the presence of arabinose and galactose was determined by thin-layer and gas chromatography (manuscript in preparation). Phosphogalactan (P-Gal) was obtained from Dr. M. E. Slodki, United States Department of Agriculture, Peoria, IL. It was isolated from Sporobolomyces sp. NRRL Y-6493 and had a hexose/phosphate ratio of 36.7. Sporobolomyces sp. galactans are lightly acetylated phosphorylated galactans with approximately equal amounts of α1,6- and α1,3-galactosyl linkages. Polymer phosphate is present exclusively in the form of α-D-galactopyranosyl-1-phospho-6′-galactosyl phosphodiester end groups (4). Recently, Dr Slodki has found upward of 15% glucose content in these phosphogalactans (personal communication, 1972). Larch arabinogalactan (AG-Lch) was purchased from K & K Labs, Plainview, NY. This carbohydrate contains multiple β-D (1 → 6) linked D-galactopyranose side chains (7). Myeloma proteins UPC 10 and JEPC 15 were purchased from Bionetics Inc., Bethesda, MD.

Results. The precipitin curves in Fig. 1 indicate that T191B not only precipitates with AG-Lch as previously reported by Potter *et al.* (3), but reacts even more strongly with AG-Tim and with P-Gal.

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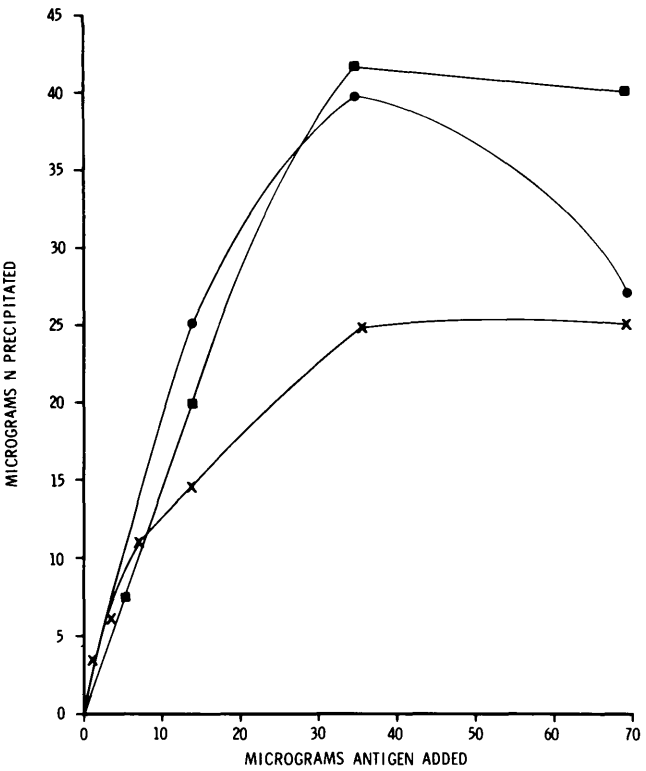

FIG. 1. Precipitation of T191B IgA myeloma protein by (×) Larch Wood Arabinogalactan (AG-Lch); (■), a phosphogalactan from Sporobolomyces sp. (P-Gal) and (●), an arabinogalactan isolated from Timothy pollen (AG-Tim). T191B serum diluted 1:8; volume of serum 70 μl, total volume per tube 100 μl.

The maximum amount of N precipitated by AG-Lch from 70 μl of a 1:8 dilution of T191B serum was 25 μg, as compared with 39 μg precipitated by AG-Tim and 43 μg precipitated by P-Gal. In additional experiments not reported here we also found that precipitation of T191B with an optimal amount of P-Gal or an optimal amount of AG-Tim removed all of the antibody precipitable by AG-Lch. Precipitation of T191B with an optimal amount of AG-Lch however, removed only 58% of the antibody precipitable by P-Gal. Since the precipitation of T191B by P-Gal was unexpected, we also added this polysaccharide to myeloma proteins UPC 10 and JEPC 15 which have a specificity for fructosan and phosphorylcholine, respectively (8). No precipitation occurred.

In Fig. 2 we demonstrate that β methyl galactoside was a potent inhibitor of the precipitation of T191B myeloma protein with AG-Lch (50% inhibition by 0.015 moles) and also inhibited the precipitation of T191B and P-Gal to a lesser extent (50% inhibition by 0.1 moles). Alpha methyl galactoside, however, was a very poor inhibitor for both polysaccharides (5–15% inhibition by 0.01 to 0.1 moles).

The data in Table I indicate that extracts from every weed or grass tested precipitated with T191B. In view of our isolation of arabinogalactans from both ragweed (9) and Timothy which precipitate with T191B, we feel safe in concluding that such polysaccharides are constituents of many or perhaps all weed and grass pollens.

Discussion. On the basis of the data presented it is evident that two additional sources of environmental antigens which might stimulate myeloma precursor cells are (1) pollens in the atmosphere, containing

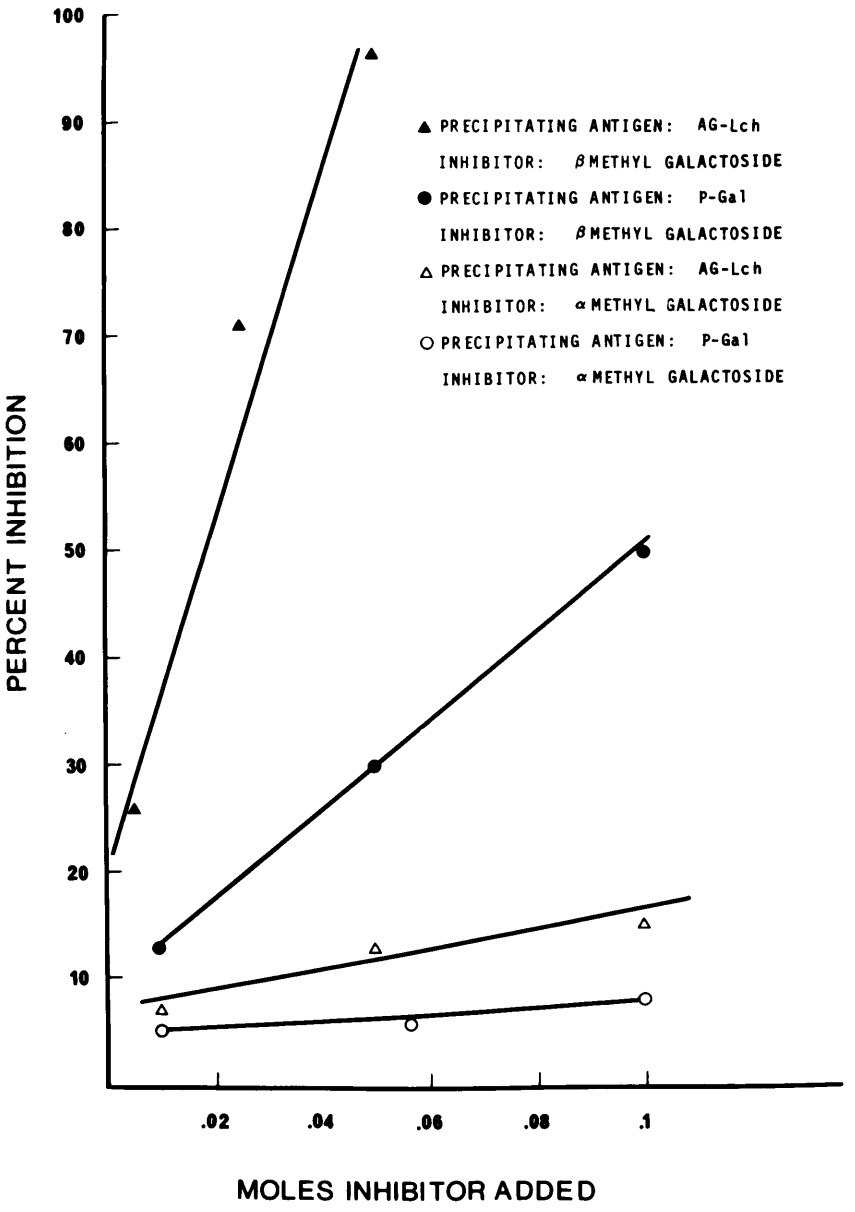

FIG. 2. Inhibition by α methyl galactoside and β methyl galactoside of precipitation of T191B myeloma protein by Larch arabinogalactan and a galactan from Sporobolomyces sp.

arabinogalactans in their cell walls, and (2) phosphogalactan from Sporobolomyces species. Sporobolomyces are yeasts which are abundant on dead and old leaves of cereals such as wheat, barley, and oats (10). Phosphogalactans from Sporobolomyces are thus likely present in mouse food containing these cereals. Pollen grains in the atmosphere have been shown to sensitize mice (11).

Of particular interest is our finding that T191B precipitates P-Gal which is an α-linked galactan. Previous studies have indicated that T191B and five other murine IgA myeloma immunoglobulins which are

TABLE I. PRECIPTATION OF T191B BY EXTRACTS FROM GRASS AND WEED POLLENS.

Pollen (20% w/v extract)	Precipitation of T191B[a]
Ragweed[b, d]	+
Timothy[c, d]	+
Bermuda[d]	+
Sage[d]	+
Red Top[d]	+
Rye Grass[d]	+
Birch	–
Beech	–
Marsh Elder	–
Hemp	–

[a] Extract (0.025 ml) added to 0.025 ml of T191B serum; volume was adjusted to 2 ml with isotonic saline and mixture incubated at 37°C for 15 min and overnight at 4°C.

[b] Precipitation also occurred with an arabinogalactan isolated from ragweed (9).

[c] Precipitation also occurred with an arabinogalactan isolated from Timothy (manuscript in preparation).

[d] No precipitates formed when these pollens were added to myeloma proteins UPC 10 or JEPC 15 which have a specificity for fructosan and phosphoryl choline, respectively (8).

galactan specific interact with β-D (1 → 6) linked D-galactopyranose residues. T191B thus appears to have a wider range of saccharide-binding specificity than had been believed. In this regard, we should mention the fact that the precipitation of hog A and H blood group substance by $RCAA_{II}$ a lectin from *Ricinus communis* could be inhibited equally well by α- and β-linked methyl D-galactopyranosides (12). In addition, precipitation of soybean agglutinin by human cyst precursor substance polysaccharides can be inhibited by both α and β glycosides of 2-acetamido-2-deoxy-D-galactose and by α and β glycosides of D-galactose (13). The lectin of *Sophora japonica* is also inhibited by both α and β D-galactopyranosides (14).

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