Shared Antigens between Mycobacterium bovis (BCG) and Other Bacterial Species

Abstract. *Antigens from* Mycobacterium bovis *and* Listeria monocytogenes *bind to serums from normal rabbits as well as from those immunized with unrelated bacteria, especially intracellular parasites. Binding is to the Fab portion of immunoglobulin G and is immunologically specific.*

Animals that have been immunized with mycobacterial cells or components have increased resistance to infection with a variety of unrelated pathogenic bacteria, especially facultative intracellular parasites such as *Listeria monocytogenes, Brucella abortus,* and *Salmonella typhimurium* (*1–3*). Enhanced resistance to tuberculous infection following immunization to unrelated bacteria has also been observed (*3–5*). These protective effects have been attributed to nonspecific, nonimmunologic mechanisms (*2–6*). Various mycobacteria share antigenic components with closely related microorganisms such as nocardiae, corynebacteria, and actinomycetes (*7*); and *L. monocytogenes* has been found to have antigenic components in common with other Gram-positive organisms (*8*). In the above-mentioned studies agglutination and precipitation tests were used to detect antibodies. In our study, we used tests that measured the primary interaction between antigen and antibody (*9*) to determine whether mycobacteria and some taxonomically unrelated bacteria share or have cross-reacting antigenic components that might account in part for apparently "nonspecific" resistance to infections.

Mycobacterium bovis strain BCG (Bacillus Calmette Guerin) was sonified and injected into rabbits as described (*10*). The following organisms were grown, killed with heat, and washed: *L. monocytogenes, B. abortus, S. typhimurium, Escherichia coli, Neisseria catarrhalis,* and *Staphylococcus epidermidis.* The washed cells were disrupted by sonification for 5 minutes, taken up in incomplete Freund's adjuvant (IFA), and injected intramuscularly into rabbits. Three injections each containing 1 mg of nitrogenous material were given at intervals of 3 weeks. A control group of six rabbits was injected with heterologous serum albumins (bovine, sheep, or human) in IFA, and another group of three rabbits was injected with IFA alone. Serums were collected from all animals before immunization and 1 week after the final injection. An additional control group received no injections, and the serums obtained at similar intervals were designated normal rabbit serums.

The sonicated BCG and *L. monocytogenes* cells were subjected to ultracentrifugation, and the resulting supernatants were treated with 0.05*M* manganese chloride to precipitate nucleic acids (*11*). These supernatants were dialyzed and then labeled with ^{125}I (*12*), and diluted so that the nitrogen content ranged from 0.1 to 0.2 μg/ml.

Antiserum binding of ^{125}I-labeled test antigens were studied by precipitating ^{125}I-labeled antigen–antibody complexes with goat antiserum to rabbit IgG as described (*10*), except that 0.1-ml portions of serums diluted 1 : 5 in borate buffer and 0.1 ml of ^{125}I-labeled antigen preparations were used. Binding by serums from immunized animals was compared with their matching normal initial serum because binding to these test antigens by serums from different nonimmunized animals varied considerably. Differences in the binding capacity to the radioactive antigens are expressed as the ratio of the number of counts per minute in the precipitates resulting from the final bleedings to the number of counts per minute from the corresponding initial bleedings. A ratio of 1.4, which represents a 40 percent increase in binding, was selected to indicate significant increased binding between the serums taken before and after immunization. Data from these experiments are presented in Fig. 1, A and B.

Serums from the animals immunized with sonicated BCG had increased binding to ^{125}I-labeled BCG test antigen, as expected. Serums from animals immunized with heterologous albumins and IFA alone and serums from nonimmunized animals showed only trace differences between initial and final bleedings. Of the animals immunized with sonicated intracellular parasitic bacteria, there was increased binding to the ^{125}I-labeled BCG test antigen by all serums from the six animals immunized with *L. monocytogenes,* four of the five immunized with *B. abortus,* and four of five of those immunized with *S. typhimurium* (Fig. 1A). Serums

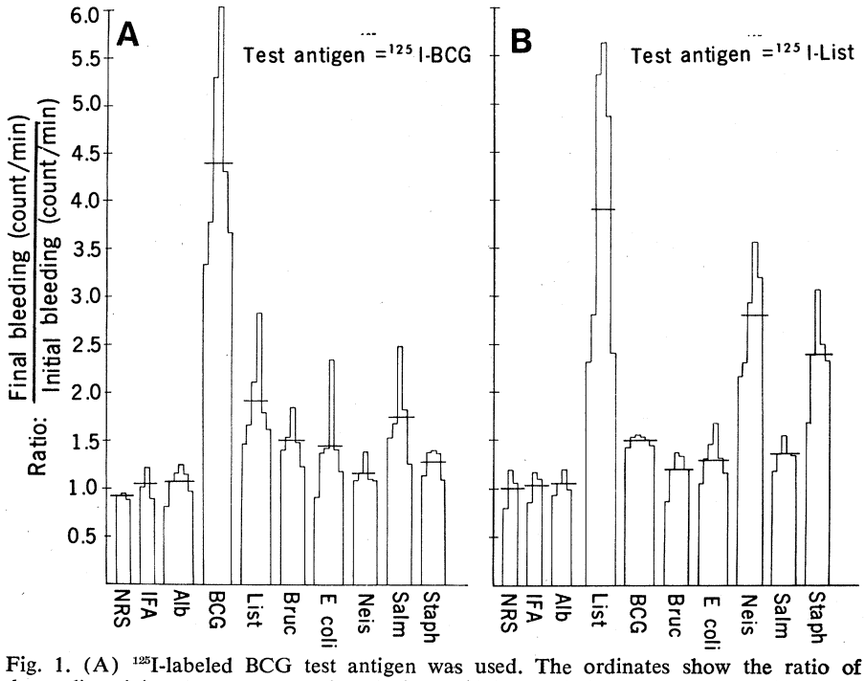

Fig. 1. (A) ^{125}I-labeled BCG test antigen was used. The ordinates show the ratio of the radioactivity (counts per minute) in precipitates derived from serums of final bleedings to that in the serums obtained before the animals were immunized. Serums were obtained from normal rabbits (NRS), rabbits immunized with incomplete Freund's adjuvant (IFA), heterologous albumins (Alb), *Mycobacterium bovis* strain BCG (BCG), *Listeria monocytogenes* (List), *Brucella abortus* (Bruc), *Escherichia coli* (E. coli), *Neisseria catarrhalis* (Neiss), *Salmonella typhimurium* (Salm), and *Staphylococcus epidermidis* (Staph). (B) ^{125}I-labeled antigen from *L. monocytogenes* was used.

Table 1. Inhibition of binding of ^{125}I-labeled test antigens from BCG and *L. monocytogenes* (List).

Reaction tested		Unlabeled BCG (μg of nitrogen)	Inhibition ratio*	Unlabeled List (μg of nitrogen)	Inhibition ratio*
^{125}I-antigen	Antibody to				
BCG	NRS †	93	0.60	423	0.45
BCG	BCG	77	0.29	423	0.77
BCG	List	93	0.56	334	0.57
List	NRS	570	0.69	70	0.61
List	BCG	210	0.60	43	0.47
List	List	570	0.89	70	0.64

* The ratio of the numbers of counts per minute in the inhibited preparation to the number of counts per minute in the noninhibited preparation. † NRS, serum obtained from normal rabbits.

from three of six animals immunized with sonicated *E. coli* also showed increased binding, but no serums from animals immunized with sonicated *N. catarrhalis* or *S. epidermidis* showed increases.

With ^{125}I-labeled test antigen from *L. monocytogenes* there was the expected increased binding by the serums from animals immunized with sonicated *L. monocytogenes* (Fig. 1B). Control serums showed no changes. Serums from all animals immunized with BCG had significant increases. None of the serums from animals immunized with *B. abortus* showed significant increases, and only one of five serums from animals immunized with *S. typhimurium* and only two of six serums from animals immunized with *E. coli* showed significant increases. All serums from animals immunized with *N. catarrhalis* and *S. epidermidis* showed increases. These observations were confirmed by radioimmunoelectrophoresis and radioimmunodiffusion (*13*).

Inhibition studies confirmed the specificity of these reactions. Antiserums were first incubated with unlabeled homologous and heterologous test antigens as well as with a variety of potentially inhibitory substances. Portions of the same antiserums were simultaneously incubated with equivalent volumes of borate buffer. Labeled test antigens were added 24 hours later to all mixtures. Binding by antiserums containing unlabeled test substances was compared to binding by control antiserums. Results are expressed as the inhibition ratio, which compares the number of counts per minute of the inhibited to the noninhibited preparations. A ratio of less than 1.0 indicated that unlabeled substances were occupying sites that would otherwise be binding labeled antigen. Representative data are shown in Table 1. Reactions were inhibited by relatively small amounts of unlabeled homologous test antigens with serums from rabbits injected with sonicated BCG or *L. monocytogenes*. In addition, binding by serums from nonimmunized rabbits was inhibited, an indication that normal rabbit serum as well as serum from immunized rabbits contained antibodies to both test antigens and that each of the labeled test antigens shared antigenic components. Heterologous unlabeled antigens produced significant inhibition only in concentrations higher than needed by homologous antigens, suggesting that only a small portion of the homologous labeled antigens was shared by the hetrologous antigens. Unlabeled *E. coli* partially inhibited binding of antiserums to the ^{125}I-labeled antigen from BCG but not to the ^{125}I-labeled *Listeria* antigen. No inhibition was produced by endotoxin (100 μg, Difco Labs), polyvinyl pyrrolidone (PVP) [5 percent solution of PVP (BDH Chemical), 44,000 and 700,000 molecular weight], bovine serum albumin [1 mg (BSA, Armour)], 2×10^7 sheep red cells either in suspension or sonicated, or samples of culture mediums (Proskauer-Beck and tryptose phosphate, Difco).

Because protein A from *Staphylococcus aureus* is known to bind to the Fc fragment of rabbit IgG even in the absence of antibodies to protein A (*14*), we undertook to determine whether the binding observed in our study was binding to the Fab antigen-combining portion of IgG. The IgG prepared by DEAE chromatography (*15*) of serums from normal rabbits and from antiserum to BCG and antiserum to *L. monocytogenes* was digested with pepsin (*16*) to produce Fab'_2. Radioimmunoelectrophoresis, radioimmunodiffusion, and precipitation of labeled antigen–Fab'_2 complexes by antiserum to IgG confirmed that Fab'_2 from normal serums, antiserum to BCG, and antiserum to *L. monocytogenes* bound labeled BCG and *Listeria* antigens.

Our studies suggest that some of the binding by normal rabbit serums to BCG as well as the binding by serums from nontuberculous humans to mycobacterial components (*17*) may be due to nonmycobacterial immunogens. The possibility should be considered that resistance to tuberculous infections may be partly due to immune mechanisms, whether cellular or humoral, that have been stimulated by nonmycobacterial organisms.

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