

This document only includes an excerpt of the corresponding thesis or dissertation. To request a digital scan of the full text, please contact the Ruth Lilly Medical Library's Interlibrary Loan Department (rlmlill@iu.edu).

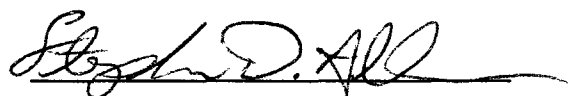
ANTIBODIES TO PEPTIDOGLYCAN DIFFERENTIATE BETWEEN CELL WALL
FRAGMENTS OF GRAM-NEGATIVE AND GRAM-POSITIVE BACTERIA

Donna Swank Rudiger

Submitted to the faculty of the University Graduate School
in partial fulfillment of the requirements
for the degree
Master of Science
in the Department of Pathology
Indiana University

May 1998

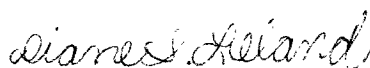
Accepted by the Graduate Faculty, Indiana University, in partial fulfillment of the requirements for the degree of Master of Science

A handwritten signature in black ink, appearing to read "Stephen D. Allen", written over a horizontal line.

Stephen D. Allen, M.D.

A handwritten signature in black ink, appearing to read "Raoul S. Rosenthal", written over a horizontal line.

Raoul S. Rosenthal, Ph.D.

A handwritten signature in black ink, appearing to read "Diane S. Leland", written over a horizontal line.

Diane S. Leland, Ph.D.

ABSTRACT

We propose that peptidoglycan (PG), a uniquely bacterial macromolecule common to virtually all bacteria, might serve as a marker for differentiation of PG from Gram-negative bacteria (GNB) vs. PG from Gram-positive (GPB). Enzyme immunoassay (EIA) was employed to characterize rat polyclonal antibodies (Pab; predominantly IgG) directed against soluble macromolecular, extensively-*O*-acetylated PG (*S-O*-PG) purified from *Neisseria gonorrhoeae* strain FA 19. Evidence that the principal epitope recognized by the Pab depended on repeating glycosidically-linked monomeric subunits was provided in previous experiments in which the addition of muramidase destroyed any reactivity, and that LPS, chitin, and chitotriose did not bind Pab. Homologous *S-O*-PG inhibited the antibody activity with doses achieving 50% inhibition (ID_{50}) of approximately 15 ng/well. Using two lots of rat sera, ID_{50} s of 18 GNB ranged from 0.08 μ g/well to 711 μ g/well with a median of approximately 15 μ g/well; ID_{50} s of the GPB ranged from 0.215 μ g/well to indeterminate ($>10^5$ μ g/well) with a median of 174 μ g/well. As expected, solubilization of PGs enhanced the reactivity. Interestingly, even though the glycan chain of most bacteria is identical, Pab differentiated between the PGs of GNB and GPB, i.e., 23 of 29 (79%) of a variety of GNB yielded insoluble PG which had an $ID_{50} < 60$ μ g/well, compared to only 7 of 25 (28%) of GPB ($p < 0.01$). Thus, antibodies to the glycan chain may be influenced by the composition and structure of the amino acids, *O*-acetylation, or degree of cross-linking of peptide chains, known differences between the PGs of GNB and GPB.

TABLE OF CONTENTS

IMPORTANCE OF THE BACTERIAL CELL SURFACE	1
COMPONENTS OF CELL WALLS	3
STRUCTURE OF THE PEPTIDOGLYCAN (PG)	9
GRAM STAIN REACTION	15
CLASSIFICATION USING PG	18
CLINICAL SIGNIFICANCE	23
IMMUNOGENICITY OF PG	25
HYPOTHESIS AND OVERVIEW	33
MATERIALS AND METHODS	35
RESULTS	44
DISCUSSION	55
REFERENCES	61