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The major outer membrane proteins of enterobacteriaceae. Their immunological relatedness and their possible role in bacterial opsonization

Hofstra, Harmen

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Chapter X

Summary

Gram-negative bacteria belonging to the family *Enterobacteriaceae* are a major cause of serious infections in hospitalized patients. Normally, these bacteria reside harmlessly as members of the host's indigenous gut flora. However, they can become potent opportunistic pathogens during periods of impaired host defense. Besides the continuous development of new antimicrobial agents and the further refinement of measures for infection prevention, many laboratories study the possibility of immunotherapy and immunoprophylaxis against Gram-negative bacteremia. However, immunological approaches to the problem of enterobacterial infections are seriously limited by the broad serotypical diversity among these bacteria. This stimulated the search for common antigens of *Enterobacteriaceae*, i.e. antigens which are shared by serotypically unrelated bacteria. This thesis deals with immunological investigations of the major outer membrane proteins of the *Enterobacteriaceae* as a new group of enterobacterial common envelope antigens, and with some aspects of the possible role of antibodies, prepared against these proteins, in host defense mechanisms.

In chapter I a brief review is given on the recent literature concerning the prevention of Gram-negative bacteremia, the prevalence of common enterobacterial antigens and the structure of the enterobacterial cell envelope.

Chapter II deals with the isolation and purification of the major outer membrane proteins of *Escherichia coli* O26 K60. Their immune response in rabbits was investigated after intravenous and intramuscular administration of the purified antigens. In addition some methods for the determination of the antibody titers and their specificity were compared. It was found that intramuscular administration of the antigens with Freund's complete adjuvant resulted in high titers of antibodies, which could be determined quickly and with a high reproducibility by the enzyme-linked immunosorbent assay (ELISA).

In chapters III to VI the results are given of immunochemical investigations on the antigenic cross-reactivity of the major outer membrane proteins of several different serotypes of *E. coli* and other *Enterobacteriaceae* species. Three proteins were found immunologically related among all enterobacterial strains studied: 1, The native trimer of the peptidoglycan-associated protein (porin), which *in vivo* is responsible for the passive diffusion of small molecules through the outer membrane; 2, The chromatographically purified and denatured porin monomer, which showed hardly any relationship with the native trimer; and 3, The non-peptidoglycan-bound OmpA protein or protein II*. Murein lipoprotein has already been described by others as an antigen shared by many different *Enterobacteriaceae*. This was confirmed in our experiments. In experiments with isolated human granulocytes we investigated the enhancement of the phagocytosis and intracellular killing of bacteria that had been pre-opsonized by antibodies against outer membrane proteins. This 'opsonic activity' of the antisera was tested by determining the total number of viable bacteria and the number of viable intracellular bacteria in mixtures of granulocytes and pre-opsonized bacteria (chapter VII) and, in addition, by determining the granulocyte's chemiluminescence response after the addition of pre-opsonized bacteria (chapter VIII). It was found that

antibodies directed against porin trimer able to enhance the phagocytosis of *E. coli*. In experiments, the opsonic activity of the antibodies was found to be higher than the activity of serotype-specific antibodies. The OmpA lipoprotein showed no opsonic activity. Chapter IX gives a general discussion. In addition, more, the results of studies concerning the effect of antibodies are given (these results are not included in this thesis). It was found that active immunization of mice with antisera did not protect them against infection with high doses (>LD100) of most of the tested encapsulated bacteria escape from being killed by antibodies against major outer membrane proteins.

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Gram-negatieve bacteriën behorende tot de familie *Escherichia coli*, zijn de voornaamste veroorzakers van infecties tijdens hun verblijf in het ziekenhuis. Het gebruik van antimicrobiële therapie en preventieve maatregelen, blijft ook het gebied van de immunotherapie en immunoprophylaxe of immunotherapie in het gebied van de schijnbaar onbepaalde verscheidenheid van serotype-antigenen, die we bij de ontwikkeling van type-specifieke vaccins tegen het spectrum. Dit stimuleert vele laboratorische werken van vaccins bereid uit, of anderszins schappelijke, d.w.z. kruisreagerende antigenen. De naamste eiwitten uit de buitenmembranen van de gemeenschappelijke antigenen en worden de activiteit van de, tegen deze eiwitten

In het eerste hoofdstuk wordt een overzicht gegeven betreffende de frequentie en de prevalentie van Gram-negatieve bacteriën met een verminderde weerstand. Verscheidene *Enterobacteriaceae* behandeld.

In het tweede hoofdstuk wordt de isolatie van de buitenmembraan eiwitten (de z.g. 'major outer membrane proteins') van verschillende serotypes van *E. coli* en andere *Enterobacteriaceae* beschreven. De immunrespons van deze eiwitten op intraveneuze en intramusculaire toediening werd onderzocht. Het bleek, dat intramusculaire toediening met adjuvant resulteerde in een hogere antititer. Het oecbaar resultaat kon worden bepaald door de ELISA Assay).

In de hoofdstukken III, IV, V en VI worden de verschillende immunochemische technieken

antibodies directed against porin trimers, porin monomers and the OmpA protein are able to enhance the phagocytosis of *E. coli* by granulocytes. In chemiluminescence experiments, the opsonic activity of the anti-outer membrane protein sera was lower than the activity of serotype-specific antiserum. Antibodies directed against murein lipoprotein showed no opsonic activity, nor did non-immune rabbit serum.

Chapter IX gives a general discussion on the results compiled in this thesis. Furthermore, the results of studies concerning the active and the passive protection of mice are given (these results are not included as a separate chapter in this thesis). It was found that active immunization of mice with the purified proteins or passive immunization with antisera did not protect the mice against intraperitoneal challenge with high doses (>LD100) of most of the bacterial strains tested. It seems that especially encapsulated bacteria escape from being opsonized by antibodies directed against the major outer membrane proteins.

Samenvatting

Gram-negatieve bacteriën behorende tot de familie *Enterobacteriaceae*, zoals bijv. *Escherichia coli*, zijn de voornaamste veroorzakers van ernstige infecties bij patiënten tijdens hun verblijf in het ziekenhuis. Naast de voortdurende ontwikkelingen op het gebied van de antimicrobiële therapie en naast de steeds verdere verfijning van preventieve maatregelen, blijft ook het onderzoek naar de mogelijkheden van immunoprofylaxe of immunotherapie in het geval van enterobacteriële infecties actueel. De schijnbaar onbeperkte verscheidenheid aan onderling niet of nauwelijks verwante, z.g. serotype-antigenen, die we bij de *Enterobacteriaceae* aantreffen, verhindert de ontwikkeling van type-specifieke vaccins of antisera met meer dan een uiterst smal spectrum. Dit stimuleert vele laboratoria tot het onderzoeken van de beschermende werking van vaccins bereid uit, of antisera bereid tegen enterobacteriële gemeenschappelijke, d.w.z. kruisreagerende antigenen. In dit proefschrift worden de voornaamste eiwitten uit de buitenmembraan van de *Enterobacteriaceae* gedefinieerd als gemeenschappelijke antigenen en worden voorts enkele aspecten van de antibacteriële activiteit van de, tegen deze eiwitten opgewekte antilichamen behandeld.

In het eerste hoofdstuk wordt een kort overzicht gegeven van recente literatuur betreffende de frequentie en de preventie van enterobacteriële infecties bij patienten met een verminderde weerstand. Vervolgens wordt de bouw van de celwand bij de *Enterobacteriaceae* behandeld.

In het tweede hoofdstuk wordt de isolatie en de zuivering van de voornaamste buitenmembraan eiwitten (de z.g. 'major proteins') van *Escherichia coli* O26 K60 beschreven. De immuunrespons van deze eiwitten werd onderzocht bij konijnen d.m.v. intraveneuze en intramusculaire toediening. Voorts werden enkele methoden vergeleken ter bepaling van de titer en de specificiteit van de gevormde antilichamen. Het bleek, dat intramusculaire toediening van de gezuiverde eiwitten met Freund's compleet adjuvant resulteerde in een hoge antilichaamtiter, die snel en met een reproduceerbaar resultaat kon worden bepaald in ELISA (Enzyme-Linked Immuno-Sorbent Assay).

In de hoofdstukken III, IV, V en VI wordt weergegeven, hoe met behulp van verschillende immunochemische technieken de immunologische verwantschap werd on-