Int. Archs Allergy appl. Immun. 77: 118–120 (1985)

0020–5915/85/0772–0118 $ 2.75/0

Role of Bacterial Adherence and Toxin Production from *Escherichia coli* on Leukotriene Generation from Human Polymorphonuclear Granulocytes

W. König[a], *J. Scheffer*[a], *K.D. Bremm*[a], *J. Hacker*[b], *W. Goebel*[b]

[a] Lehrstuhl Medizinische Mikrobiologie und Immunologie, Arbeitsgruppe Infektabwehr, Ruhr-Universität, Bochum;
[b] Institut für Mikrobiologie, Universität Würzburg, FRG

Introduction

The role of bacteria and bacterial toxins in inducing lipoxygenase factors and leukotrienes with chemotactic (LTB_4) and spasmogenic (LTC_4, D_4 E_4) properties has been recently suggested. Among the bacterial toxins streptolysin O, α-toxin, lipase and enterotoxin from *Staphylococcus aureus*, leukocidin from *Pseudomonas aeruginoa*, θ-toxin from *Clostridium perfringens* proved to be potent inducers of leukotriene generation from various human cells [6–8]. It is well established that bacterial colonization is a prerequisite of bacterial infection [1, 2, 4, 5, 9–12, 16, 17]. In order to analyze the role of bacterial adhesion and toxin production on leukotriene formation, genetically cloned bacteria which differed in their pili structures were analyzed [13, 14].

Material and Methods

Human polymorphonuclear granulocytes (PMNs) were purified and incubated with various bacterial strains. The following strains were analyzed; *Escherichia coli* K12-MS^+, Hly^-; *E. coli* pHly 152-MS^+, Hly^+, *E. coli* pANN 202-312, MS^+, Hly^+, subclone of pHly 152, *E. coli* pANN 5311-MS^+, Hly^+, *E. coli* pANN 5411-MS^+, Hly^+, *E. coli* 536, MS^+ MR^+ (type Vb), Hly^+, *E. coli* 536/21-MS^+, MR^-, Hly^-, *E. coli* 536/31-MS^+, MR^+, Hly^-, *E. coli* 536/21 pANN 5211-MS^+, MR^-, Hly^+, *E. coli* 536/21 pANN 5311-MS^+, MR^-, Hly^+; *E. coli* 536/31 pANN 5211 MS^+, MR^+, Hly^+; *E. coli* 536/31 pANN 5311 MS^+, MR^+, Hly^+. Leukotrienes were analyzed by reversed-phase HPLC or radioimmunoassay for LTC_4; histamine by the automated fluorometric analyzer; lipoxygenase activity by the generation of 5-HETE and 5-HPETE from ^{14}C arachidonic acid, γ-glutamyltranspeptidase and dipeptidase activities on incubation of cell-free supernatants with synthetic LTC_4 and LTD_4, respectively; the degree in hemolysin production photometrically by the lysis of erythrocytes at 530 nm. For the chemotactic assay either human neutrophils or guinea pig eosinophils were used as target cells in the Boyden chamber [7].

Results

Human PMNs (1×10^7/ml) were incubated with the various *E. coli* K12 and *E. coli* 536 strains. The luminol- and lucigenin-dependent chemiluminescence was recorded. Bacterial strains exhibiting MS^+ pili induced significant chemiluminescence when these strains also produced hemolysin. In *E. coli* 536 strains, which in addition to MS^+ pili also express MR^+ pili, higher chemiluminescence was recorded with the MR^+ as compared to the MS^+, MR^- strains; the activity even exceeded that obtained with the MS^+, MR^- but hemolysin-producing strains. These results indicated that the respiratory burst of granulocytes is efficiently modulated by the type of adherence and degree of toxin production. When the rate of phagocytosis versus adherence was examined with ^3H-thymidine-labeled bacteria phagocytosis was higher with MS^+-adherent strains as compared to MS^+, MR^+ bacteria. The presence of MR^+ pili even inhibited the phagocytosis. These data were also supported by experiments in which opsonized bacteria with the above described expression of pili were studied.

Incubation of human PMNs with the various bacterial strains led to the release of chemotactic activity for human neutrophils and guinea pig eosinophils when washed bacteria or the culture supernatants of the late logarithmic phase were used as stimuli. A further analysis of these results was obtained by studying the release of 5-HETE, LTB_4, LTC_4, LTD_4 and LTE_4. *E. coli* K12 (MS^+, Hly^-) induced a slight release of LTB_4 and a significant generation of 5-HETE, but insignificant amounts of LTC_4. With washed Hly^+-producing strains, a dose- and time-related release of LTC_4 was observed. The culture supernatant proved to be more active in inducing LTB_4 and LTC_4 release as compared to the washed bacteria. *E. coli* 536 (MS^+,

Table I. Summary of results. Histamine relesase and generation of leukotrienes from human PMNs on interaction with various strains of *E. coli*

E. coli strain	Rat mast cells: histamine, ng		PMN: LTC_4, ng		PMN: LTB_4, ng	
	bact.	sup.	bact.	sup.	bact.	sup.
K 12 (Hly^-/Fim^+)	0	19	< 0.1	< 0.1	< 1.0	< 1.0
K 12 pHly 152 (Hly^+/Fim^+)	66	37	6.5	19.0	< 1.0	8.1
K 12 pANN 202-312 (Hly^+/Fim^+)	5	37	< 0.1	9.0	< 1.0	13.8
K 12 pANN 5211 (Hly^+/Fim^+)	75	48	5.2	19.8	< 1.0	18.6
K 12 pANN 5311 (Hly^+/Fim^+)	116	59	< 0.1	10.0	2.6	5.9
K 12 pANN 5411 (Hly^+/Fim^+)	140	48	3.1	17.5	5.2	10.0

Table II. Summery of results. Induction of chemiluminescense (CL), histamine release and generation of LTC^4 and hemolysin

E. coli	*Hemolysin*, △ OD/min	PMN: CL (luminol), cpm × 10^5		Bact. + mast cells histamine, ng	Bact. + PMN: LTC_4, ng
		bact.	sup.		
536 (Hly^+, Fim^+)	0.018	1.25	1.25	107	< 0.1
536/21 (Hly^-, Fim^-)	0	0.05	0.01	10	< 0.1
536/31 (Hly^-, Fim^+)	0	0.1	0.03	10	< 0.1
536/21 pANN 202/312 (Hly^+, Fim^-)	0.001	0.02	0.01	3	< 0.1
536/21 pANN 5211 (Hly^+, Fim^-)	0.013	0.05	0.95	7	2.2
536/21 pANN 5311 (Hly^+, Fim^-)	0.012	0.02	1.25	27	4.6
536/31 pANN 5211 (Hly^+, Fim^+)	0.015	2.6	1.2	49	11.0
536/31 pANN 5311 (Hly^+, Fim^+)	0.017	0.25	1.40	77	28.5

MR^+, Hly^+), *E. coli* 536/21 (MS^+, MR^-, Hly^-), *E. coli* 536/31 pANN 5311 (MS^+, MR^+, Hly^+) induced significant amounts of LTC_4 when the bacterial supernatant or, in the latter case, whole bacteria were analyzed. MS^+, MR^+, Hly^+ bacteria and their culture supernatant elicited LTB_4, C_4, D_4 and E_4 release when the supernatants were used as stimuli while the Hly^-, MS^+ or Hly^-, MS^+, MR^+ bacteria induced significant amounts of 5-HETE. Leukotriene release was more pronounced with strains which had MS^+, MR^+ pili as compared to MS^+, MR^- pili, i.e. an inhibition of phagocytosis by MR^+ pili apparently enhances the release of leukotrienes. The concomittant stimulation of cells with the ionophore in the presence of *E. coli* K12 (MS^+, Hly^-) reduced the formation of LTB_4 significantly; in the presence of the hemolysin-producing strain, LTB_4 release was highly enhanced. When histamine release was studied with the various *E. coli* K12 and *E. coli* 536 strains the following results were obtained: washed Hly^+ bacteria were more active as compared to Hly^- bacteria; a similar pattern was observed when the culture supernatant was analyzed. These data suggested that histamine release is markedly affected by toxin-producing bacteria. Our results show that adherence as well as toxin production modulate the cellular responsiveness for leukotriene release (table I, II).

We recently presented evidence that various endotoxins and lipid A are potent inducers of LTC_4 release from human granulocytes. Endotoxin sensitive and less sensitive donor cells were described. In addition, various bacterial exotoxins were able to trigger cells for leukotriene release. Among them the thiol-activatable toxins (streptolysin O, alveolysin from *Bacillus alvei* and θ-toxin from *C. perfringens*) were more precisely analyzed. These toxins induced leukotriene formation, lipoxygenase and dipeptidase release. Thus, studies on arachidonic acid metabolites and histamine release prove to be a sensitive model to analyze membrane biochemical interactions of various bacterial strains and to provide a rapid determination of their potential pathogenicity. The described models could be useful in determining the early stages of bacterial colonization versus bacterial infection. Indeed,

in the classical model of heavily burnt patients significant levels of leukotrienes (C_4, E_4) were detected in plasma, while the blood cultures proved to be negative up to the death of the patients.

References

1 Alouf, J.E.: Bacterial toxins: an outlook. Toxicon *20:* 211 (1982).

2 Beachey, E.H.: Bacterial adherence. Recept. Recogn., B *6:* (Chapman and Hall, London and New York, 1980).

3 Berger, H.; Hacker, J.; Juarez, A.; Hughes, C.; Goebel, W.: Cloning of the chromosomal determinants encoding hemolysin production and mannose-resistent hemagglutination in *Escherichia coli.* J. Bact. *152:* 1241 (1982).

4 Bernheimer, A.W.: Interaction between membranes and cytolytic bacterial toxins. Biochim. biophys. Acta *344:* 27 (1974).

5 Blumenstock, E.; Jann, K.: Adhesion of piliated *Escherichia coli* strains to phagocytosis: differences between bacteria with mannose-sensitive pili and those with mannose-resistant pili. Infect. Immunity *35:* 264 (1982).

6 Bremm, K.D.; Brom, H.J.; König, W.; Bohn, A.; Theobald, K.; Bhakdi, S.; Lutz, F.; Fehrenbach, F.J.: Bacteria and bacterial exotoxins induce leukotriene formation from human polymorphonuclear granulocytes. Monogr. Allergy, vol. 18, p. 196 (Karger, Basel 1983).

7 Bremm, K.D.; Brom, J.; König, W.; Spur, B.; Crea, A.; Bhakdi, S.; Lutz, F.; Fehrenbach, F.J.: Generation of leukotrienes and lipoxygenasefactors from human polymorphonuclear granulocytes during bacterial phagocytosis and interaction with bacterial exotoxins. Zentbl. Bakt. Abt. I Orig. B. Hyg. *254:* 500 (1983).

8 Bremm, K.D.; Brom, J.; Alouf, J.W.; König, W.; Spur, B.; Crea, A.; Peters, W.: Generation of leukotrienes from human granulocytes by alveolysin from bacillus alvei. Infect. Immunity *44:* 188 (1984).

9 Duguid, J.P.; Old, D.C.: Adhesive properties of enterobacteriaceae. Recept. Recogn., B *6:* 187 (1980).

10 Fehrenbach, F.J.; Schmidt, C.M.; Huser, H.: Early and late events in streptolysin O induced hemolysis. Toxicon *20:* 223 (1982).

11 Freter, F.; Jones, G.W.: Models for studying the role of bacterial attachment in virulence and pathogenesis. Rev. infect. Dis. *5:* 647 (1983).

12 Gaastra, W.; Graaf, F.K. de: Host-specific fimbrial adhesins of noninvasive enterotoxigenic *Escherichia coli* strains. Microbiol. Rev. *46:* 129 (1982).

13 Goebel, W.; Hedgpeth, J.: Cloning and functional characterization of the plasmid-encoded hemolysin determinant of *Escherichia coli.* J. Bact. *151:* 1290 (1982).

14 Hacker, J.; Knapp, S.; Goebel, W.: Spontaneous deletions and flanking regions of the chromosomally inherited hemolysin determinant of *E. coli* 06-strain. J. Bact. *154:* 1145 (1983).

15 Hagmann, W.; Keppler, D.: Leukotriene antagonists prevent endotoxin lethality. Naturwissenschaften *69:* 594 (1982).

16 Jann, K.; Schmidt, G.; Blumenstock, E.; Vosbeck, K.: *Escherichia coli* adhesion to *Saccharomyces cerevisiae* and mammalian cells: role of piliation and surface hydrophobicity. Infect. Immunity. *32:* 485 (1981).

17 Jones, G.W.; Isaacson, R.E.: Proteinaceous bacterial adhesins and their receptors. CRC crit. Rev. Microbiol. *10:* 229 (1983).

18 König, W.; Kunau, H.W.; Borgeat, P.: Comparison of the eosinophil chemotactic factor with endogeneous hydroxyeicosatetraenoic acids; in Samuelsson, Paoletti, International symposium on leukotrienes and other lipoxygenase products, p. 301 (Raven Press, New York 1982).

19 Samuelsson, B.: The leukotrienes: a group of biologically active compounds including SRS-A. Trends pharmacol. Sci. *19:* 227 (1980).

Correspondence to: Dr. W. König,
Lehrstuhl Medizinische Mikrobiologie und Immunologie,
Arbeitsgruppe für Infektabwehrmechanismen,
Ruhr-Universität Bochum,
Universitätsstr. 150,
Postfach 10 21 48,
D-4630 Bochum 1 (FRG)