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INABILITIES AS AN IMMUNOADJUVANT OF CELL WALLS OF THE GROUP B PEPTIDOGLYCAN TYPES AND THOSE OF ARTHROBACTERS

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SUMMARY The cell walls from several bacterial species whose peptidoglycans are the group B types (Schleifer and Kandler) and those of two arthrobacters were shown to be inactive or only weakly active as an immunoadjuvant in both induction of delayed-type hypersensitivity and stimulation of circulating antibody levels to ovalbumin when administered to guinea pigs as a water-in-oil emulsion, in sharp contrast to the adjuvant-active cell walls of the group A peptidoglycan types which were previously studied. The possible reason for the inabilities as an adjuvant of these cell walls was discussed in relation to the chemical structures of peptidoglycans.

INTRODUCTION

In the first paper of this series (Kotani et al., 1975), we reported that the cell walls from all 21 species of gram-positive bacteria whose peptidoglycans were the group A types (Schleifer and Kandler, 1972) were adjuvant-active in both stimulation of serum antibody levels and induction of delayed-type hypersensitivity, with two exceptions, when administered with ovalbumin to guinea pigs as a water-in-oil emulsion. Exceptions were the cell walls from *Micrococcus lysodeikticus* (NCTC 2665) and *Staphylococcus epidermidis* (ATCC 155). A later study has revealed that *S. epidermidis* cell walls, though adjuvant-inactive in themselves, gained distinct immunoadjuvant activities if they were solubilized by digestion with endo-*N*-acetylmuramidase or cross-bridge splitting endopeptidase (unpublished observation).

In the present study we examined the immunoadjuvancies of cell walls of bacterial species whose peptidoglycans are the group B types (Schleifer and Kandler, 1972) and those from

two arthrobacters whose peptidoglycans are the group A type, but shares with adjuvant-inactive *M. lysodeikticus* cell walls a peculiarity of the peptidoglycan structures, namely the substitution of the α-carboxyl group of the D-glutamic acid residue by amino acids or the amides of amino acid, but not ammonia.

MATERIALS AND METHODS

1. *Cell walls*

Test cell wall specimens were prepared from the following bacterial species as described in previous papers cited in parenthesis: *Microbacterium lacticum* ATCC 8280 (Schleifer et al., 1968), *Eubacterium limosum* (*Butyribacterium rettgeri*) ATCC 10825 (Miller et al., 1966), *Corynebacterium poinsettiae* NCPP 177 and *Corynebacterium insidiosum* NCPP 1110 (Perkins and Nieto, 1970; Perkins, 1971)., *Corynebacterium betae* NCPP 373 (Perkins, 1965a), *Arthobacter atrocyaneus* ATCC 13752 (Interschick et al., 1970), *Arthrobacter* sp. NCIB 9423 (Fiedler et al., 1970), and *Ampullariella regularis* (Perkins, 1965b).

2. *Assay of the immunoadjuvant activities*

The methods previously described (Kotani et al., 1975) were essentially followed except that 1/75 M phosphate buffered saline (pH 7.0) was used instead of saline solution to preparae water-in-oil emulsion.

RESULTS

The results of assay of the immunoadjuvant activities of test cell wall preparations are summarized in Table 1, with structural characteristics of each of their peptidoglycans. All of the test cell walls, except those of *A. atrocyaneus*, were found to be inactive in both adjuvant activities to induce delayed-type hypersensitivity and to stimulate circulating antibody

TABLE 1. *Inabilities of cell walls of the group B peptidoglycan types and of those from arthrobacters guinea pigs (test dose 100 μg)*

No. of experimental group	Test species (strain)	Corneal response (48 hr) Mean (Range)	Skin response (48 hr) Erythema (mm) Mean±S.E.[a]	Skin response (48 hr) Induration (Ratio) Mean±S.E.[b]	Antibody level (Ratio) Mean±S.E.[c]
43	*Microbacterium lacticum* (ATCC 8280)	0.3 (0 −1.0)	10 ± 0.97	1.5 ± 0.12	1.3 ± 0.51
43	*Eubacterium limosum* (ATCC 10825)	0	7 ± 1.7	1.3 ± 0.19	0.24 ± 0.05
43	*Corynebacterium poinsettiae* (NCPP 177)	0	6 ± 0.17	1.5 ± 0.17	0.65 ± 0.13
44	*Corynebacterium betae* (NCPP 373)	0	10 ± 0.58	1.5 ± 0.07	0.94 ± 0.31
45	*Corynebacterium insidiosum* (NCPP 1110)	0.3 (0 −1.5)	8 ± 1.1	1.7 ± 0.21	1.5 ± 0.35
44	*Arthrobacter atrocyaneus* (ATCC 13752)	1.4 (0.5−3.0)	11 ± 2.4	1.6 ± 0.12	0.79 ± 0.66
44	*Arthobacter* sp. (NCIB 9423)	0.5 (0 −1.0)	8 ± 1.5	1.6 ± 0.06	0.86 ± 0.39
44	*Ampullariella regularis*	0	10 ± 0.75	1.5 ± 0.05	0.77 ± 0.31
43	None (FIA control)	0	9 ± 1.3	1.6 ± 0.13	[62 ± 20.3][d]
44	〃 〃	0.3 (0 −1.0)	9 ± 0.13	1.7 ± 0.26	[101 ± 65.1]
45	〃 〃	0.3 (0 −1.0)	9 ± 0.52	1.6 ± 0.10	[85 ± 38.8]

[a] Average diameter of redness (mm).

[b] Ratio of double thickness of the skin injected with 100 μg ovalbumin/0.1 ml saline to that of the skin of the opposite side.

levels to ovalbumin when administered to guinea pigs as a water-in-oil emulsion. The cell walls from *A. atrocyaneus* exhibited a weak adjuvant activity in induction of delayed-type hypersensitivity, but was found inactive in stimulation of serum antibody levels at the present experimental conditions.

The walls of *A. regularis* were also found to be inactive in the immunoadjuvancy, though there has not been enough information on chemical structures of the peptidoglycans of this organism.

DISCUSSION

The cell wall peptidoglycans whose immunoadjuvant activities were assayed in this study are known to be different from those of the usual group A peptidoglycan types in the following points (Fig. 1): with the cell walls of the group B peptidoglycan types, 1) amino acid (R_4) linked to muramic acid is glycine or L-serine, but not L-alanine, and 2) the cross linkages containing a diamino acid are involved in the α-carboxyl group of the D-glutamic acid or *threo*-3-hydroxyglutamic acid (R_3) in one peptide subunit and the carboxyl group of the C-terminal D-alanine (R_1) in the neighboring peptide subunit, and with the cell walls from two arthrobacters belonging to the group A peptidoglycan type, on the other hand, they are characterized by the fact that the α-carboxyl group of the D-glutamic acid (R_3) is substituted by glycine amide or D-alanine amide, unlike the usual group A type peptidoglycans where the α-carboxyl group of the D-glutamic acid is amidated (Schleifer and Kandler, 1972).

The study has been performed to find the reasons for the observed inabilities as an im-

to induce delayed-type hypersensitivity and to stimulate serum antibody levels to ovalbumin in

IgG_2	Characteristics of peptidoglycan structures					(cf. Fig. 1) Peptidoglycan types
Mean (Range)	R_4	R_3	R_x	Cross linkage	R_2	
0	Gly	D-Dlu (Hyg)[e]		$R_3 \rightarrow Gly_{1-2} \xrightarrow{\alpha}$ L-Lys $\leftarrow R_1$	L-Lys (L-Hsr)[e]	B1α
0	L-Ser	D-Glu		$R_3 \xrightarrow{\varepsilon}$ D-Lys $\leftarrow R_1$ $R_3 \xrightarrow{\delta}$ D-Orn $\leftarrow R_1$	L-Orn	B2α
0	Gly	D-Glu		$R_3 \rightarrow$ (Gly) $\xrightarrow{\delta}$ D-Orn $\leftarrow R_1$	L-Hsr[e]	B2β
0	〃	〃		〃	〃	〃
0.2 (0–1.0)	Gly	D-Glu (Hyg)[e]		$R_3 \xrightarrow{\gamma}$ D-Dab $\leftarrow R_1$	*N*-Ac-L-Dab[e]	B2γ
0	L-Ala	D-Glu	Gly-$CONH_2$	$R_2 \xleftarrow{\varepsilon}$ L-Ser $\leftarrow$ L-$Ala_{2-3} \leftarrow R_1$	L-Lys	A3α
0.1 (0–0.5)	L-Ala	D-Glu	D-Ala-$CONH_2$	$R_2 \xleftarrow{\varepsilon}$ L-Thr $\leftarrow$ L-Ala $\leftarrow R_1$	L-Lys	A3α
0						
0						
0						
0						

[c] Ratio of antibody nitrogen (μg/ml serum) in the test group to that in the respective FIA control group.
[d] μg Antibody nitrogen/ml serum specimen.
[e] Hyg, *threo*-3-hydroxyglutamic acid; Hrs, homoserine; *N*-Ac-Dab, N^{γ}-acetyldiaminobutyric acid.

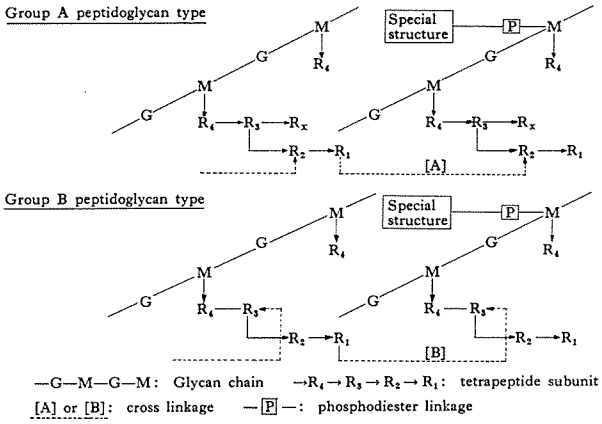

FIGURE 1. *A schematic presentation of the chemical structures of cell walls of group B and group A peptidoglycan types [Kato, 1975]*

munoadjuvant of the cell wall preparations examined here, by use of synthetic muramyl peptides. The results will be reported elsewhere.

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