Purification and characterization of a saliva-interacting cell-wall protein from *Streptococcus mutans* serotype f by using monoclonal-antibody immunoaffinity chromatography

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A rat monoclonal antibody, LO SM2, of the immunoglobulin M class, specific for a saliva receptor (SR) from *Streptococcus mutans* serotype f, was able to precipitate the SR from crude cell-wall-associated antigens (WEA) of this bacteria in presence of a detergent mixture. We have then used the technique of monoclonal-antibody immunoaffinity chromatography to purify the *S. mutans* SR. Pure SR was obtained from a crude WEA fraction with a single chromatographic step. The active SR could be eluted from the column in a highly purified form with 0.2M-glycine/HCl, pH 2.8. The final yield was about 32% in terms of binding activity. Characterization of the SR by crossed immunoelectrophoresis, sodium dodecyl sulphate- or 4–30%-native-gradient-polyacrylamide-gel electrophoresis showed that the receptor is a single polypeptide chain of M_r approx. 74000. Native or denaturated forms of the SR adsorbed on to a solid support, such as nitrocellulose, are recognized by monoclonal antibody LO SM2, and both forms are still able to bind the ligand, saliva.

A series of observations indicate that *Streptococcus mutans* binding to the tooth surface in the oral cavity is a two-step reaction, involving initial attachment of cells followed by co-aggregation with other bacterial species (Clark & Gibbons, 1977; Kuramitsu & Ingersoll, 1977). Adherence of *S. mutans* seems to be mediated by salivary glycoproteins, which are selectively adsorbed on to the hydroxyapatite of tooth enamel and form the acquired pellicle (Hamada & Slade, 1980). Recent studies suggested that ionic and hydrophobic interactions may be important binding mechanisms holding the bacteria to the pellicle (Gibbons & Etherden, 1983; McBride *et al.*, 1984). However, the exact nature of the molecules responsible for adherence, on the *S. mutans* cell surface, has not yet been clearly identified. It has been suggested that lipoteichoic acids and some surface proteins are the components responsible for the surface hydrophobicity of these bacteria (Rölla, 1976; Weerkamp & Jabos, 1982).

Abbreviations used: WEA, cell-wall-associated antigens; SDS, sodium dodecyl sulphate; e.l.i.s.a, enzyme-linked immunosorbent assay.

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Recently, a second mechanism implicated in bacterial adherence has been identified for *S. sanguis*, consisting of lectin-like interactions between streptococcal adhesins and salivary glycoproteins (McBride & Gisslow, 1977; Ericson & Rundegren, 1983). Such a phenomenon could also be implied in saliva-induced streptococcal aggregation or co-aggregation. Since saliva-mediated bacterial aggregation has been reported to promote either bacterial accumulation on the tooth (Gibbons & Van Houte, 1980) or bacterial elimination from the oral cavity by blocking attachment sites (Gibbons & Van Houte, 1975), it seems of great importance to study the bacterial receptors for saliva.

Numerous proteins exist within the cell wall and extend from the cell surface (Russell, 1979). Some of these proteins were characterized as saliva receptors (SR) of the *S. mutans* cell wall, especially two proteins of 135 and 125 kDa, which have a glycoprotein nature (Ogier *et al.*, 1984). Previously described monoclonal antibodies to *S. mutans* cell-wall components (F. Ackermans, J. P. Klein, F. Cormont & H. Bazin, unpublished work) were found to react with protein 125 kDa. We therefore used one of these monoclonal antibodies to purify

and characterize one SR from *S. mutans* serotype f by monoclonal-antibody immunoaffinity chromatography.

Materials and methods

Saliva

Unstimulated human whole saliva was collected from ten individuals in ice-chilled plastic containers and pooled. The saliva was heat-inactivated for 30 min at 56°C and then clarified by centrifugation for 20 min at 12000g.

Bacteria and antigen preparation

S. mutans OMZ 175 (serotype f) strain (from Dr. B. Guggenheim, Dental Institute, University of Zürich, Zürich, Switzerland) was maintained in brain/heart infusion broth (Difco Laboratories) and crude cell-wall-associated antigens (WEA) were extracted from the bacterial pellet with 0.5M-phosphate buffer, pH 6.0, as previously described (Schöller *et al.*, 1981). Protein concentration was determined by the procedure of Lowry *et al.* (1951), with bovine serum albumin as standard.

Antiserum production

Rabbit anti-(whole saliva) and rat anti-(*S. mutans* serotype f WEA) sera were prepared as previously described by Ogier *et al.* (1984). The IgG fractions from both sera were purified by chromatography on DEAE-Trisacryl M (IBF) essentially by the method of Corthier *et al.* (1984).

Monoclonal-antibody production and purification

LO SM2, an IgM monoclonal antibody produced in rats against a 125 kDa saliva-binding protein from *S. mutans* serotype f, was prepared (F. Ackermans, J. P. Klein, F. Cormont & H. Bazin, unpublished work). Ascites fluids were made by intraperitoneal injection of hybridoma cells into rats. The LO SM2 antibody was purified from ascites fluid by precipitation against 0.1M-Tris/HCl buffer, pH 8.0, and gel filtration on Bio-Gel A5m (Bio-Rad) (Cisar *et al.*, 1981). Fractions eluted from this column containing pure antibody (determined by agarose-gel electrophoresis) were used to prepare the immunoaffinity column.

Production of monoclonal-antibody immunoaffinity column

CNBr-activated Sepharose-4B (Pharmacia) was reconstituted, washed and then coupled to LO SM2 antibody (8–10 mg of protein/ml of gel) according to the manufacturer's instructions. The beads (30 ml) were packed into a column and equilibrated with 0.01M-Tris/HCl (pH 7.2)/0.15M-NaCl (TS buffer).

Affinity purification

WEA from *S. mutans* serotype f was used as starting material for purification of the saliva receptor. Usually approx. 30 mg of WEA in TS buffer containing 1% Triton X-100 and 0.05% SDS were applied on the antibody column. The column was washed with TS buffer until the A_{280} returned to the baseline value. The specifically bound material was eluted with either 5M-NaCl or 0.2M-glycine/HCl, pH 2.8, and was immediately neutralized with 1M-Tris. After removal of the eluting agent by dialysis against TS buffer at 4°C, the samples were analysed by electrophoresis in the presence of SDS or in 4–30% polyacrylamide gradient gels.

Measurement of saliva-binding activity

The binding activity of the purified SR was determined by using a competitive e.l.i.s.a. A 0.05 ml portion of an appropriate *S. mutans* suspension in 1M-NaHCO₃, pH 9.6 (equivalent to 4×10^8 bacteria/ml) was allowed to adsorb to each well of a microtitre plate (Nunc) overnight at 37°C. After the removal of the coating solution and five washings of the wells with TS buffer containing 0.05% Tween 20 (TST buffer), 0.025 ml of serial dilutions of purified SR was added in each well. Then 0.025 ml of a saturating amount of saliva was added to the wells and allowed to incubate for 2 h at room temperature.

After the plates were washed five times with TST buffer, 0.05 ml of rabbit anti-saliva IgG was added and incubated for 1 h at room temperature. The plates were washed again with TST buffer and 0.05 ml of alkaline-phosphatase-labelled goat anti-rabbit IgG (Miles Laboratories) was added to each well and incubated for 1 h as described above. After five washes, 0.05 ml of 0.0038M-*p*-nitrophenyl phosphate in 1M-diethanolamine, pH 9.8, was added and the plates were read at 405 nm, after 1 h, in a Multiskan reader (Flow Laboratories). The minimal concentration of saliva required for saturation of *S. mutans* binding sites was initially determined by varying the amounts of saliva added in the test system. The percentage inhibition of saliva binding to bacteria was calculated as:

$$\frac{A_{405}^{\text{Control}} - A_{405}^{\text{SR}}}{A_{405}^{\text{Control}}} \times 100$$

One unit of binding activity is defined as the amount of SR which allows a 50% inhibition of saliva binding in the standard test.

Polyacrylamide-gel electrophoresis

SDS/10%-(w/v)-polyacrylamide-gel electrophoresis, performed by the method of Laemmli (1970), was used to check purity and estimate

protein subunit size. The gels were stained with AgNO_3 (Merril *et al.*, 1982). Native M_r was determined by electrophoresis to equilibrium under non-denaturing conditions on 4–30%-polyacrylamide gradient-gel slabs (Pharmacia) in 0.09M-Tris/0.08M-boric acid buffer, pH 8.3, for 4 h at a constant 300 V at 10°C. Protein was detected by Coomassie Blue staining. Carbohydrate-containing components were stained with periodic acid/Schiff reagent by the procedure of Fairbanks *et al.* (1971).

Detection of receptor by protein blotting

The Western-blotting technique described by Towbin *et al.* (1979) was used. The nitrocellulose sheets were then incubated with either peroxidase-labelled rat polyclonal anti-WEA IgG or monoclonal anti-(125 kDa protein) LO SM2, followed by mouse peroxidase-conjugated IgG1 monoclonal antibodies against rat K-light chains (MARK-1) (Bazin *et al.*, 1984) and enzyme substrate. Saliva binding to the receptor was performed as described by Ogier *et al.* (1984).

Immunoprecipitation

Sample of WEA (100 µg), adjusted to 2% (v/v) Triton X-100 and 0.01% (w/v) SDS, were incubated with LO SM2 antibody (200 µg) for 1 h at 4°C, followed by addition of MARK-1-Sepharose CL-4B beads (30 min, 25°C). The samples were then treated as previously described by Ogier *et al.* (1984) to generate Western blots. The antigens were detected by incubating the paper with peroxidase-labelled rat anti-WEA IgG.

Immuno-electrophoresis

Crossed immuno-electrophoresis was performed by the method of Weeke (1973). Samples were analysed in 1% (w/v) agarose (IBF) gels of 1.5 mm thickness on gel-bond plates (100 mm × 100 mm; Pharmacia). The gel contained 1% (v/v) Triton X-100 and barbital buffer, pH 8.6 (I.02). The first dimension was run at 2.5 V/cm for 2 h and the second dimension at 1.5 V/cm for 17 h. The immunoplates were stained with Coomassie Blue after washing and drying.

Immuno-electron microscopy procedure

Immuno-electron microscopy was carried out by using bacteria (2×10^9) suspended in TST buffer and monoclonal antibody (LO SM2) added to a final concentration of 10 µg/ml, followed by incubation for 1 h at 4°C with rocking. After incubation, the bacteria were washed three times with TST buffer and labelled by the indirect technique using peroxidase-conjugated MARK-1 (2.5 µg/ml). The bacteria were then processed as previously described (Schöller *et al.*, 1983) and examined in a

Philips EM 200 electron microscope without additional staining. No peroxidase labelling of cell-surface components was observed when bacteria were allowed to react with TST buffer or control IgM followed by MARK-1-peroxidase.

Labelling of antibodies

MARK-1-, rat anti-WEA IgG- and rabbit anti-saliva IgG-peroxidase conjugates were prepared as described by Avrameas & Guilbert (1971). MARK-1 was coupled to CNBr-activated Sepharose 4B as described above for monoclonal antibody LO SM2.

Chemical modification of SR

Periodate treatment of WEA or SR was conducted as previously described (Ogier *et al.*, 1984), with NaIO_4 at a final concentration of 0.02M.

Results

One of us (F. Ackermans, unpublished work) previously isolated four hybridoma cell lines secreting monoclonal antibodies which bound to a 125 kDa cell-surface saliva receptor (SR) from *S. mutans* serotype f. The cell lines were derived by the fusion of rat IR 983F cells with spleen cells from rats immunized with the unfractionated cell-wall WEA from *S. mutans* serotype f. When whole cells of *S. mutans* were incubated with the monoclonal antibodies (LO SM1, LO SM2, LO SM3 and LO SM4), lysed in Triton X-100 and antigen-antibody complexes were immunoprecipitated with MARK-1-Sepharose, a cell-wall SR 125 kDa protein as well as a 76 kDa protein were present in the immunoprecipitates (Fig. 1, lane 2). These antibodies also apparently bound to both proteins after separation of the WEA proteins by electrophoresis in SDS/polyacrylamide gels and transfer to nitrocellulose sheets. Monoclonal antibody LO SM2 was chosen for characterization of the 125 kDa SR from *S. mutans* serotype f.

The specificity of antibody LO SM2 was further examined by immunoprecipitation of soluble WEA in presence of Triton X-100 and SDS, which did not interfere with the antigen-antibody reaction (Ogier *et al.*, 1984). As shown in Fig. 1 (lane 3), two proteins having M_r values of about 125 000 and 76 000 could be precipitated from *S. mutans* crude WEA fraction. If the WEA fraction was submitted to NaIO_4 oxidation before immunoprecipitation, only the low- M_r band of M_r 76 000 was present (Fig. 1, lane 4). Antibody LO SM2 also bound only to the 76 kDa protein in immunoblots of NaIO_4 -pretreated WEA. Identical results were obtained with WEA fraction resolved in 4M-urea/SDS-containing gels before electrophoretic transfer of the protein to nitrocellulose.

Fig. 1. Specificities of antibody LO SM2 determined by immunoprecipitation procedures

Sample of *S. mutans* whole cells or WEA extracts were analysed as described in the Materials and methods section. The lanes shown are: (2) 2×10^{10} cells incubated with LO SM2 (100 μ g), followed by lysis with TS buffer containing Triton X-100 and SDS; (3) 100 μ g of soluble WEA treated with Triton X-100 and SDS, precipitated with monoclonal antibody LO SM2 and (4) 100 μ g of soluble WEA pretreated with 0.02M-NaIO₄, precipitated with monoclonal antibody LO SM2. The antigen-antibody complexes were recovered with MARK-1-Sepharose, separated by electrophoresis in SDS/10%-polyacrylamide gels and transferred to nitrocellulose paper. The proteins adsorbed on the blots were incubated with peroxidase-labelled rat anti-WEA IgG. A silver-stained WEA fraction resolved in SDS/10%-polyacrylamide gel is shown in (1). M_r values for the two major components detected by the antibody (arrows) were determined by reference to protein standards indicated on the left.

Since soluble 125 kDa SR forms a stable complex with monoclonal antibody LO SM2 in the presence of Triton X-100 and SDS, we theorized that the 125 kDa SR, contained in the crude WEA from *S. mutans*, would bind to immobilized monoclonal antibodies in presence of this detergent mixture. The WEA fraction, adjusted to 1% Triton X-100 and 0.05% SDS, was directly loaded on to the LO SM2-Sepharose column. The unbound material was eluted with TST buffer. Elution of the putative SR was accomplished with one of the following reagents: 5M-NaCl or 0.2M-glycine/HCl, pH 2.8. A slightly better recovery yield of SR could be obtained after elution of the antibody column with glycine/HCl buffer (Table 1). The use of a low-pH

buffer for protein elution was compatible with an excellent retention of binding activity (Table 1) and was routinely used as a more efficient eluting agent. Assuming the total activity initially applied to the LO SM2 Sepharose to be 100%, most (63%) of the activity did not bind to the antibody and appeared in the TST washes.

Therefore WEA included a subpopulation of SR (27–31%) that could be purified by affinity chromatography on a LO SM2-monoclonal-antibody-Sepharose column. The existence of other types of SR in the WEA fraction was indirectly demonstrated by further tests, which showed the absence of reactivity of unbound material contained in the TST washes with monoclonal antibody LO SM2. A second chromatography of the TST washes on the affinity column did not show any retention of binding activity. The fact that SR recognized by LO SM2 antibody was removed from the TST fraction was also confirmed by immunoprecipitation and crossed immunoelectrophoresis. No additional binding activity could be removed from the TST washes after addition of LO SM2 antibody followed by MARK-1 Sepharose as second antibody. That the SR was removed from the TST fraction and not inactivated was also confirmed by crossed immunoelectrophoresis with LO SM2 antibody (results not shown).

Fig. 2(a) shows that the SR eluted from the immunoabsorbent column gave only one precipitin arc in antigen-antibody crossed immunoelectrophoresis, as compared with the reference pattern, which showed at least 11 precipitin arcs (Fig. 2b). Fig. 3(a) (lane 1) shows a SDS/10%-polyacrylamide-gel electrophoresis of the affinity-purified SR. One silver-stained band of approx. M_r 74000 was found under denaturing conditions. Electrophoresis of the SR, under non-denaturing conditions, on a 4–30%-polyacrylamide gradient gel, again revealed one band with an apparent native M_r of 72000 (Fig. 3b, lane 1), indicating that the SR was a monomer. Antibody LO SM2 bound to the purified receptor in its denatured (Fig. 3a, lane 2) or native (Fig. 3b, lane 2) form in immunoblots. Unlike the 125 kDa SR present in the WEA, the purified receptor did not stain with the periodic acid/Schiff reagent. On the other hand, NaIO₄ oxidation of the purified receptor had an effect neither on its electrophoretic mobility nor on its recognition by antibody LO SM2.

Affinity purified SR was resolved either by SDS/polyacrylamide-gel or by native 4–30%-polyacrylamide-gradient-gel electrophoresis and transferred to nitrocellulose paper. The blots were then incubated with saliva, followed by peroxidase-labelled anti-saliva IgG to assess whether the receptor adsorbed on the nitrocellulose reacted with saliva. Both denatured (Fig. 3a, lane 3) and

Table 1. Purification of SR component by affinity chromatography

	Protein (mg)	Binding activity* (units)	Specific activity (units/mg of protein)	Purification (fold)	Yield (%)
Crude extract	30	375	12.5	1	100
TST-buffer washes	26	236	9.1	0.72	62.9
Elution with:					
5M-NaCl	0.26	101.4	390	31.2	27.1
0.2M-Glycine/HCl	0.29	116	400	32	30.9

* The binding activity was determined as described in the Materials and methods section after dialysis against TST buffer to remove NaCl or glycine/HCl.

Fig. 2. Crossed immunoelectrophoresis of crude and purified SR against rat anti-(*S. mutans* WEA) IgG. Purified (a; 0.5 µg of protein) or crude (b; 15 µg of protein) SR were subjected to electrophoresis into agarose in the first dimension and to immunoelectrophoresis into agarose containing 1% (v/v) rat anti-WEA IgG in the second dimension. Immunoprecipitates were stained with Coomassie Blue. The anode is at the top right.

native (Fig. 3b, lane 3) forms of the receptor bound the ligand. Also, saliva did not react with M_r marker proteins present in high concentrations (Figs. 3a and 3b, lane 4), confirming the specificity of the interaction with the receptor. These results also indicate that the saliva binding to the receptor is not mediated by the glycosidic moieties present in the 125 kDa protein.

The localization of the SR on the bacterial cell was further examined by immunoelectron-microscopy procedures on thin sections of *S. mutans* serotype f. Monoclonal antibody LO SM2 reacted only with components located on the exterior of the bacterial cells, thus confirming the cell-surface localization of the 74 kDa saliva-binding protein (Fig. 4).

Discussion

Immunoaffinity chromatography using monoclonal antibodies has been successfully used for

purification of several antigens (Baillyes *et al.*, 1982; Denny *et al.*, 1982). These presented a great advantage over polyclonal antibodies. Since a monoclonal antibody recognized only one epitope on an antigen, binding of an antigen to such a column should, in principle, be weaker than to one made with polyclonal antibodies, and antigen should be eluted in the active form more easily. In the present paper we described a simple procedure for the purification of a *S. mutans* SR from extracts in which this receptor represents only a very small percentage of the total protein, by using a monoclonal-antibody immunoaffinity chromatography.

The specificity of antibody LO SM2 was originally determined by e.l.i.s.a., immunoblotting of SDS/polyacrylamide-gel-electrophoresis-resolved WEA antigens, or immunoprecipitation of whole cells. From these procedures, antibody LO SM2 appears to be specific for two components that migrated into a region containing the 125 kDa saliva-binding protein and a 76 kDa protein (Ogier

Fig. 3. Polyacrylamide-gel electrophoresis and Western-blotting analysis of affinity-purified SR

Polyacrylamide-gel electrophoresis of SR eluted from a LO SM2-Sepharose column and subsequent transfer to nitrocellulose paper were performed in either SDS/10%-polyacrylamide gels (a) or 4–30%-polyacrylamide non-denaturing gradient gels (b) as indicated in the Materials and methods section. Lane 1(a), purified SR stained with AgNO_3 ; lane 1(b), Coomassie Blue-stained SR; Lane 2, blotting of denatured (a) or native (b) SR with LO SM2. Ligand blotting of denatured (a) or native (b) SR (lane 3) or M_r markers (lane 4). The blots were incubated with saliva diluted 1:2 in TST buffer, followed by peroxidase-labelled anti-saliva IgG. The migration of M_r markers is indicated.

et al., 1984). This antibody also binds to both 125 kDa and the 76 kDa protein in soluble extracts in the presence of detergent.

However, only the 76 kDa antigen is precipitated with LO SM2 antibody after treatment of the crude extract with 0.025 M NaIO_4 . This confirms preceding results that have shown a diminution of monoclonal or polyclonal antibodies binding to antigen 125 kDa (Ogier *et al.*, 1984; F. Ackermans, J. P. Klein, F. Cormont & H. Bazin, unpublished work), suggesting the presence of carbohydrate moieties in the 125 kDa antigen.

Antibody column made with LO SM2 was proved to bind efficiently the *S. mutans* protein and could be subsequently eluted with either NaCl or glycine/HCl buffer to give a good yield. The positive aspect of our isolation procedure is that the purified protein retains its specific ligand-binding activity. The binding specificity of isolated receptor for saliva was proved by the observation that both native or denatured forms of the receptor, adsorbed on to nitrocellulose, could bind the ligand. In addition, saliva binding to *S. mutans* cells could be blocked by simultaneous incubation of the purified receptor and saliva in the competitive e.l.i.s.a. test. The affinity-purified SR from *S. mutans* was resolved on SDS or native polyacrylamide-gel electrophoresis into sharp bands with apparent M_r values of approx. 74 000 and 72 000 respectively, which still reacted with

antibody LO SM2 in immunoblotting techniques. That the 74 kDa SR was the only protein recovered after elution of the affinity column argues for a dissociation of the 125 kDa SR occurring during the passage through the affinity column in presence of detergents. The addition of Triton X-100 and SDS to the crude extract is very important, since omission of the detergent mixture gives rise to a poor degree of purification of the SR, which is then recovered together with low- M_r proteins. This suggests the existence of strong interactions between the different components contained in the initial extract.

The slight difference between the electrophoretic profiles of crude or purified SR could be due to our electrophoretic conditions, giving a poor resolution of crude proteins migrating in the 76 kDa region and forming a broad band. These results are in contradiction with our previous report (Ogier *et al.*, 1984), where we found that only the 125 kDa protein binds saliva components. However, the absence of ligand binding to the 76 kDa component present in the crude extract could be explained either by the presence of only low quantities of SR in the 76 kDa region, or by a binding hindrance exerted by the other proteins present at this level. In our opinion the 125 kDa SR from *S. mutans* is an aggregate of two 74 kDa SR molecules. The exact relationship between both components remains to be elucidated, but it is

Fig. 4. Immunoelectron-microscopic detection of SR with antibody LO SM2

Freshly grown cells of *S. mutans* serotype f were incubated with monoclonal antibody LO SM2, followed by MARK-1-peroxidase and substrate as described in the Materials and methods section. The bacteria were then fixed in 2% (v/v) glutaraldehyde in TST buffer, post-fixed with 1% (w/v) OsO₄ in TST buffer and embedded in Epon. Thin sections (0.01 μm) cut on a Sorvall-Porter microtome were examined in an electron microscope without staining. The presence of precipitate on the bacterial cell surface (shown by the arrows) depicts sites of immunochemical localization of the 74kDa SR.

unlikely that the 125kDa complex could be an artefact due to the preparation method, since it is always present in numerous preparations obtained over a 2-year period.

In summary we have used the technique of monoclonal-antibody immunoaffinity chromatography in a simple purification scheme to produce active SR from *S. mutans* in good yield. Although the serotype specificities of the immobilized monoclonal antibody have not been investigated in detail, on the basis of previous results (F. Ackermans, J. P. Klein, F. Cormont & H. Bazin, unpublished work), the bound monoclonal antibody does interact with cell-surface proteins from other *S. mutans* serotypes, and extracts from these bacteria could be used as source of SR.

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References

- Avrameas, S. & Guilbert, B. (1971) *Eur. J. Immunol.* **1**, 394–396
- Baillyes, E. M., Newby, A. C., Siddle, K. & Luzio, J. P. (1982) *Biochem. J.* **203**, 245–251
- Bazin, H., Xhuderbise, L. M., Burtonhoy, G., Lebacqz, A. M., De Clercq, L. & Cormont, F. (1984) *J. Immunol. Methods* **71**, 9–16
- Cisar, O. J., Barsumian, E. L., Curl, S. M., Vatter, A. L., Sandberg, A. L. & Siraganian, R. P. (1981) *J. Immunol.* **127**, 1318–1322
- Clark, W. B. & Gibbons, R. J. (1977) *Infect. Immun.* **18**, 514–523
- Corthier, G., Boschetti, E. & Charley-Poulain, J. (1984) *J. Immunol. Methods* **66**, 75–79
- Denny, R. M., Fritz, R. R., Patel, N. T. & Abell, C. W. (1982) *Science* **215**, 1400–1403
- Ericson, T. & Rundegren, J. (1983) *Eur. J. Biochem.* **133**, 225–261
- Fairbanks, G., Steck, T. L. & Wallach, D. F. H. (1971) *Biochemistry* **10**, 2606–2617
- Gibbons, R. J. & Etherden, I. (1983) *Infect. Immun.* **41**, 1190–1196
- Gibbons, R. J. & Van Houte, J. (1975) *Annu. Rev. Microbiol.* **29**, 19–44
- Gibbons, R. J. & Van Houte, J. (1980) in *Bacterial Adherence* (Beachy, E. H., ed.), pp. 61–104. Chapman and Hall, London
- Hamada, S. & Slade, H. D. (1980) *Bacteriol. Rev.* **44**, 331–384
- Kuramitsu, H. & Ingersoll, L. (1977) *Infect. Immun.* **17**, 330–337
- Laemmli, U.K. (1970) *Nature (London)* **227**, 680–685
- Lowry, O. M., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- McBride, B. C. & Gisslow, M. T. (1977) *Infect. Immun.* **18**, 35–40
- McBride, B. C., Song, M., Krasse, B. & Olsson, J. (1984) *Infect. Immun.* **44**, 68–75
- Merril, C. R., Goldman, D. & Van Keuren, M. L. (1982) *Electrophoresis* **3**, 17–23
- Ogier, J., Klein, J. P., Sommer, P. & Frank, R. M. (1984) *Infect. Immun.* **45**, 107–112
- Rölla, G. (1976) in *Microbial Aspects of Dental Caries (Spec. Suppl. Microbiol. Abstr.)* (Stiles, H. M., Loesche, W. J. & O'Brien, T. C., eds.), vol. 1, pp. 309–324, Information Retrieval, Washington DC
- Russell, R. R. B. (1979) *J. Gen. Microbiol.* **114**, 109–115
- Schöller, M., Klein, J. P. & Frank, R. M. (1981) *Infect. Immun.* **31**, 52–60
- Schöller, M., Klein, J. P., Sommer, P. & Frank, R. M. (1983) *J. Gen. Microbiol.* **129**, 3271–3279
- Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
- Weeke, B. (1973) in *A Manual of Quantitative Immunoelectrophoresis Methods and Applications* (Axelson, N. H., Krøll, J. & Weeke, B., eds.), pp. 15–35, Universitetsforlaget, Oslo
- Weerkamp, A. H. & Jabos, T. (1982) *Infect. Immun.* **38**, 233–242