THE REACTIVE NATURE OF ACUTE RHEUMATIC FEVER: EVIDENCE FROM STREPTOCOCCAL CELL WALL ANTIGEN DETECTION BY IMMUNOTECHNOLOGY

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Aim: The life-threatening group A streptococcal (strep) infection and its sequelae, including acute rheumatic fever (ARF), re-emerged as a serious health problem. The fleeting arthritis of ARF is considered a form of reactive arthritis. However, no one has confirmed this by investigating its synovial fluid cells for a possible presence of strep cell wall antigens using western blot in humans. This is the aim of the current study.

Methods: Synovial fluid- (SF) and peripheral blood-mononuclear cells (PB-MNCs) from 40 patients with ARF and 10 patients with rheumatoid arthritis (RA), who served as a control group, were examined for strep antigens by immunofluorescence (IF) and western blot (WB) techniques using rabbit polyclonal antiserum and mouse monoclonal antibodies.

Results: Extensive bacterial cultures of SF, blood and throat were negative. By IF, a significant proportion (37.5%) of ARF samples (Chi-square=3.72, p=0.048) showed positive staining in SF- as well as PB-MNCs with both rabbit polyclonal antiserum and mouse monoclonal antibodies. Further, IF was significantly higher in ARF- than RA-patients (Mann-Whitney p=0.022) in whom we failed to observe any staining.

By immunoblotting, 21 samples from ARF patients (52.2%) were positive with mouse monoclonal antibodies specific for strep peptidoglycan-polysaccharide (PG-PS) complex in SF-Cs (a band with a molecular weight of 28 kD) and PB-MNCs (29kD) and its proportion was significant (p=0.0008). With rabbit polyclonal antiserum, significant blots (p=0.027) were noted in 27/40 ARF patients (67.5%) indicating strep PG-PS (24-29kD broad band) in SF-Cs and PB-MNCs. Blots by both mono- and poly-clonal antibodies were significantly higher (p=0.003&=0.001, respectively) than control samples that were non-reactive using both types of antibodies.

Conclusion: The reactive nature of acute rheumatic fever is suggested by the frequent detection of streptococcal cell wall antigen from affected joints using both, immunofluorescence and western blotting.

Key words: Streptococcal antigen, Rheumatic fever, Rheumatoid arthritis.

INTRODUCTION

Rheumatic fever (RF) is a catastrophic illness world-wide with an incidence between 10 and 15 million per year (1). In developed countries, worldwide, RF and life-threatening group A streptococcal (strep) infections have re-emerged to become once again a serious health threat (2). A similar situation was noted (3) in emerging nations, including Egypt (4) and the Gulf countries (5).

For ARF diagnosis, two major, or one major and two minor manifestations of Johns Criteria accompanied by supporting evidence of antecedent group A strep infection are required. The updated guidelines highlighted a subgroup of “exceptions to Jones Criteria” for patients with chorea, indolent carditis and previous history of rheumatic fever or rheumatic heart disease “RHD” (6). At the present time, echocardiography is insufficient to be the sole criterion for valvulitis in ARF. (7).
The term reactive arthritis (ReA) was first used by Ahvonen and co-workers in 1969 (8) to describe joint disease developing after infection elsewhere. Forms of ReA where bacteria have been shown to play a role include pathologic entities in association with Campylobacter (9), Salmonella (10), Chlamydia (11), Shigella (12) infections, Lyme disease (13) and Reiter’s syndrome (14).

The pathogenesis ARF-ReA (and arthralgia) is poorly understood (15) although autoimmune mechanisms after group A strep pharyngitis may be involved (16). The arthritogenicity of Strep pyogenes cell wall is attributed to an autoimmune-like disease (17) which for reasons still unclear predominantly manifests itself as ReA (18). The most virulent factor in strep cell wall is surface M protein (19) which immunologically cross-reacts with human tissues (20) including joints (21). An immunogenic factor in M proteins, peptidoglycan (PG), is covalently bound to a group specific polysaccharide (PS) and both (PG-PS) has been implicated in the pathogenesis of animal models of ReA (22).

The aim of this prospectively-designed, cross-sectional controlled study is to confirm the reactive nature of acute rheumatic fever in humans by investigating the existence of the streptococcal cell wall antigen, PG-PS, in cells from synovial fluid and peripheral blood by both immunofluorescence and western blotting.

**MATERIAL AND METHODS**

**Patients**

This study comprised of two groups of patients; group 1 40 patients with acute rheumatic fever (ARF) and group 2 ten rheumatoid arthritis (RA) patients who served as control group (Table 1). In both groups, the patients were randomly chosen from the patients’ list of the Rheumatology Clinic in the Out-patient Department.

| Parameter                  | Rheumatic Fever (n:40) | Rheumatoid Arthritis (n:10) | p value |
|---------------------------|------------------------|----------------------------|---------|
| Age, years                | 16±3.5                 | 44±12                      | 0.0001  |
| Gender, M/F               | 1:3                    | 2:5                        | Descriptive |
| Number of involved joints | 3±1                    | 11±5                       | Descriptive |
| ASO, in Todd Units        | 341±57                 | 80±21                      | 0.001   |
| CRP, µg/ml                | 42±15.3                | 46.6±18.8                  | ns      |

ASO; Antistreptolysin O titer, CRP; C-reactive protein, ns; non significant

**Figure 1.** Indirect immunofluorescent staining of the streptococcal cell wall peptidoglycan-polysaccharide complex antigen epitopes present on the synovial-fluid cells (panels A) from acute rheumatic fever patients. The panel labeled B shows negative staining from rheumatoid arthritis patients (x200).
Randomization was done manually without the use of a computer algorithm to allocate every other patient on the patients' list of either disease. The ARF patients have been diagnosed as having initial attack of ARF (15/40) on basis of the updated revised Jones’ Criteria (23) while recurrent attacks depended on history (24) in 25 out of the 40 patients with RF. Apart from the evidenced recent group A strep infection, 22/25 of patients had the classical migratory arthritis (25) while a non-migratory type of polyarthritis that responded within 72 hours to non-steroidal anti-inflammatory drugs was noticed in the remaining 3 patients (26). None of the RF patients with recurrent attacks had an ESR of less than 50 mm in the first hour. Additionally, we observed other minor criteria such as elevated CRP in all and prolonged PR interval on ECG tracing from those with carditis. Of note, 30/40 ARF patients had evidence of RHD. Also, other manifestations such as anemia, precordial chest pains, or epistaxis were variably present.

The only exclusion criterion was the presence of post-streptococcal reactive arthritis (PS-ReA) (27). The controls met the American College of Rheumatology criteria (28) for diagnosing RA and had no history of recent strep infections. The study was approved by the Local Medical Research and Ethics Committee. Informed consent was obtained from all the participants. The study was conducted according to Good Clinical Practice (GCP) guidelines.

**Specimens Collection**

Three samples were collected from ARF patients and their RA controls for the planned study; synovial fluids (SF), peripheral blood (PB) (for leukocytes, anti-streptolysin O titer [ASOT] & C-reactive protein [CRP]) and throat swabs. Slow aspiration of SF from knee joint of 32 patients; from elbow in five and from ankle joints of the remaining three patients was done. Part of SF was cultured for viable bacteria while the majority was mixed with heparin (50 IU/ml) in preparation for Ficoll Isopaque gradient centrifugation (at 200g for 10 minutes [min]) to separate mononuclear cells (MNCs). Portion of cell deposit was stored at -70°C and the remaining was suspended in Hank’s balanced salt solution, cyto-centrifuged
onto slides at 200 g for 5 min, fixed in ethanol/acetic acid at -20 °C, and stored at -70 °C to be used for western blotting (WB). At the same time, PB samples were collected and treated by Ficoll–Isopaque gradient centrifugation to separate its MNCs.

**Bacterial Cultures**

Blood and SF were cultured in an attempt to isolate the causative microorganism with special attention to optimize culture conditions for detecting even minute amount of bacteria as reported earlier (10). Sterile swab was taken from the throat and examined for gram-positive microbes and were directly inoculation on blood agar for 24 hrs and extended, if no growth was seen, to 48 hrs. Beta-haemolytic strep were searched for by its characteristic zone of haemolysis, colony morphology, and serology (Patho DX, Latex).

**Antibodies Used By Immunotechnology**

**Streptococcal cell wall antigen preparation**

We have prepared strep cell wall fragments from group A strep by using Mickle cell desintegrator as described by Salton and Horne (29). Strep cell wall fragments were then stored at -20°C.

**Rabbit Polyclonal Antiserum Preparation**

Antisera against Strep pyogenes were raised by immunization of rabbits with heat-killed bacteria. The rabbits were injected intravenously once a week for 5 days. 

*Figure 4. Examples of positive and negative control samples.*
weeks with increasing amount of bacteria (0.25, 0.5, 1.0, 1.5, and 2.0 ml of bacterial suspension containing approximately $10^9$ bacteria/ml phosphate-buffered saline [PBS]). The animals were bled 1 week after the final injection. As controls, we used the same antiserum, adsorbed extensively with the immunizing bacteria.

**Mouse Monoclonal Antibodies**

We used a commercial mouse monoclonal antibodies specific for PG-PS complex of strep cell wall (ATCC hybridomas 8515[US Pat. 4,596,769]) in this analysis. To ascertain reactivity of antibodies, two controls were used as previously described (30). The first control was the same antibodies used in patients’ specimens and the second one (positive control) was PB-MNCs from a healthy subject that were incubated with the same strep bacteria for one hr and then carefully washed.

**In-Vitro Indirect Immunofluorescence**

Rabbit antiserum to strep PG-PS was overlaid to slides and incubated at room temperature (RT) for 30 minutes (min). The slides were then washed with PBS containing 0.2% bovine serum albumin (BSA) and stained with fluoriscinated fragments of antirabbit IgG (1:200) (Sigma Chemicals, St. Louis, USA) at RT for 30 min. Slides were then washed with PBS before being examined with Leitz diplan-incidence light fluorescence microscope with an Osram HBO 100-W mercury lamp. Other slides were overlaid with mouse monoclonal antibodies specific for strep PG-PS and were then incubated and washed as described before. Lastly, they were stained with fluorencinated fragments of anti-mouse IgG (1:200) (Sigma Chemicals. St. Louis, USA) before being examined by an investigator who was blinded to the slides code.

**Electrophoresis and Western blotting (WB)**

The cell deposits were solubilized in Laemmli’s sample buffer and separated on a vertical sodium dodecyl sulfate-polyacryl-amide slab gel, with a staking gel of 5% acrylamide and a resolving-gradient gel of 5-17.5 %, or 10-12.5 % acrylamide. Standards of known molecular weight were included in each gel run (electrophoresis Low Molecular Weight Calibration Kit, Pharmacia Fine Chemicals AB, Uppsala, Sweden) (31). For WB, the separated components in the gel were immediately transferred electrophoretically onto nitrocellulose sheets (HAHY 00100; pore size, 0.45 µm/Millipore; SA, France) with the use of a Transphore apparatus (LKB-Bromma, Sweden) and pre-chilled TRIS-glycin buffer (pH 8.3) for 1.5 hrs. Molecular weight standards were visualized with amido-black staining. Non-specific binding sites of nitrocellulose were blocked by overnight incubation at 4°C in PBS (pH 7.4) containing 10% horse serum. After 5 washes with PBS, nitrocellulose sheets were cut into strips and each was stained separately and was allowed to react overnight at 4°C with a 1: 300 dilution of rabbit antiserum or with a 1: 20 dilution of mouse monoclonal antibody in PBS with 10% horse serum. They were then rewashe and incubated for three hrs at RT with horseradish-peroxidase-labeled anti-rabbit or un-conjugated rabbit anti-mouse immunoglobulins (DAKO Immunoglobulins, Copenhagen, Denmark). The mouse blots were again washed and incubated with horseradish-peroxidase-labeled anti-rabbit immunoglobulins. After three washes, the strips were developed with 4-chloro-1-naphthol (Sigma Chemicals, UK) (32).

**Statistical analysis**

Variables are expressed as mean±SD, unless otherwise stated. The chi-squared test, student t-test and Mann-Whitney test were used as appropriate. The correlation coefficient was estimated between qualitative variables by Spearman’s test. The statistical significance was established at a p value of ≤ 0.05. Non-significant level was noted as NS. The statistical analysis was performed using the Social Package for Statistical Science (SPSS) version 10.

**RESULTS**

The ARF patients were significantly younger (t-test p<0.0001) with lesser number of joints affected by arthritis compared to RA control group (3+1 vs 11+5). Likewise, the level of anti-streptolysin O titer (ASOT) in ARF was significantly higher (t-test, p<0.001) than the corresponding figure in RA patients. However, the difference in C-reactive protein (CRP) level was non-significant.
Of note, extensive bacterial cultures of SF, blood and throat were negative for serological evidence for microbial infection with any of the organisms known to cause ReA, particularly salmonella, chlamydia, shigella, or brucella.

**Immonofluorescence (IF)**

By in-vitro IF, fifteen out of our forty ARF patients (37.5%) showed strep PG-PS antigens with rabbit polyclonal antiserum as well as with mouse monoclonal antibodies in their SF- and PB-Cs. The proportion of positive results within ARF group was statistically significant (Chi-square=3.72, p=0.048) and is significantly higher than the negative IF observed in RA (Mann-Whiney p= 0.022). The observed positive staining was identical with both antibodies and was seen mostly in polymorphnuclear- and to a much lesser extent in mononuclear-cells (Figure 1, A). On the average, five to ten percent of the cells seen in the examined slides showed granular staining of cytoplasm. Negative staining was noted in samples from all RA control group (Figure 1, B).

**Western Blotting**

Both, SF- and PB-cells from 21 out of the investigated 40 patients (52.5%) with ARF were positive on WB analysis using monoclonal antibodies specific for strep PG-PS antigen. Within this group, the proportion of positive results was statistically significant (Chi-square=8.2, p=0.0008) and a significant difference between WB observed in ARF patients and the negative results seen in all RA patients was noticed (Mann-Whiney; p=0.003). The observed bands were of the same molecular weight but with varying intensities. A light band with a molecular weight of 28 kD was visualized in SF-Cs of fifteen patients who have recent ARF (Figure 2, lane 1) and a sharper band with the same molecular weight was seen in PB-MNCs of some of them (Figure 2, lane 2). In the remaining patients who had repeated attacks of ARF polyarthritis, a strong band with a molecular weight of 28 kD was visualized in SF-Cs slides (Figure 2, lane 3) as well as in PB-MNCs (Figure 2, lane 4).

Samples from 27 (67.5%) patients with ARF were positive in slides of both SF-Cs and PB-MNCs with rabbit polyclonal antiserum and this proportion was significant (Chi-square=4.9, p=0.027). Similar to our observation with monoclonal blots, the noted reactivity on using the polyclonal antiserum in ARF was much higher than the non-reactive pattern of blots in controls and the difference was statistically significant (Mann-Whitney; p= 0.0001). Interestingly, patients with arthritis only showed a band with a molecular weight of 26-29kD (Figure 3, lane 2) in their SF-Cs and PB-MNCs (Figure 3, lane 3). However, the reactivity pattern was much broader in those having arthritis in addition to carditis, (Figure 3, lane 4). Of note, Lane 1 of the same figure showed a control normal rabbit serum.

In addition, we observed a significant correlation between the positive reactivity with the application of mouse monoclonal antibodies and that with rabbit polyclonal antiserum (r=0.729, p=0.0001). We, however, failed to detect any significant correlation between the positive reactivity with either antibody and the elevated ASOT or CRP in ARF patients.

Figure 4 displayed examples of positive and negative staining of SF-C from patients with ARF. The reactivity of rabbit antiserum and monoclonal antibodies against strep pyogenes and against PB-MNCs incubated with the bacteria in vitro for one hour are also shown. With both antibodies the reactivity pattern of ingested bacteria was considerably narrower than that of native bacteria.

**DISCUSSION**

In this study we detected streptococcal (strep) antigens in synovial-fluid (SF) and peripheral-blood mononuclear cells (PB-MNCs) from patients with acute rheumatic fever (ARF) with the use of immunotechnology including both indirect immunofluorescence (IF) and western blot (WB) analysis. Despite a century of research, the pathogenesis of rheumatic fever (RF) remains incompletely understood (33, 34). One of the major obstacles to understanding the pathogenesis of RF is the inability to establish an animal model of rheumatic heart disease. However, the cytokotoxicity (enzyme-induced) theory and the immunologic (immune-mediated) theory have been proposed for such explanation. Genetic susceptibility (host factors) is present in 3-5% of people and the mode
Streptococci-triggered acute rheumatic fever.

The percentage of positive results was higher on using WB technique compared to indirect IF since it detects digested cell wall components rather than cell wall antigen epitopes. The positive results of monoclonal antibody immunoblot were considerably higher than negative results. The presence of PG component of the strep PS as a potential key player in the development of ARF was confirmed because we used monoclonal antibodies as well. However, other bacterial degradation product can not be excluded. A significant association was detected between the results of immunoblotting analyses by using polyclonal antibodies and monoclonal antibodies inferring shared epitopes by the antigens.

In conclusion, the reactive nature of acute rheumatic fever in humans may...
be considered due to our observation of microbial (streptococcal) antigens in affected joints and in peripheral blood along with absence of viable bacteria. Also, detection of these streptococcal antigens is common and would be easier with the immunotechnology.

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REFERENCES
1. Gibofsky A, Zabriskie JB. Rheumatic fever and poststreptococcal reactive arthritis. Curr Opin Rheumatol 1995;7: 299-305
2. Hosier DM, Craenen JM, Teske DW. Resurgence of acute rheumatic fever. Am J Dis Child 1981;141:730-33
3. Groves AM. Rheumatic fever and rheumatic heart disease: an overview. Rheumatic fever and rheumatic heart disease are still major causes of disease in the developing world today. Trop Doct 1999;29:129-32
4. Olivier C. Rheumatic fever: is it still a problem? J Antimicrob Chemother 2000; 45: 13-21
5. Eltohami EA, Hajar HA, Folger GM Jr. Acute rheumatic fever in an Arabian Gulf country: effect of climate, advantageous socioeconomic conditions, and access to medical care. Angiology 1997;48:481-89
6. Saxena A. Diagnosis of rheumatic fever: current status of Jones Criteria and role of echocardiography. Indian J Pediatr 2000;67: S11-S14
7. Guidelines for the diagnosis of rheumatic fever. Jones Criteria, 1992 update. Special Writing Group of the Committee on Rheumatic Fever, Endocarditis, and Kawasaki Disease of the Council on Cardiovascular Disease in the Young of the American Heart Association. JAMA 1992;268:2069-73
8. Ahvon P, Sievers K, Aho K. Arthritis associated with Yersinia enterocolitica infection. Acta Rheumatol Scan 1969;15: 232-53
9. Tiovanen A, Tiovanen P, eds. Reactive arthritis. Boca Raton, Fla.: CRC Press. 1988;1-166
10. Granfors K, Jalkanen S, Lindberg A, et al. Salmonella lipopolysaccharide in synovial cells from patients with reactive arthritis. Lancet 1990;335:685-88
11. Poole ES, Highton J, Wilkins RJ, Lamont IL. A search for Chlamydia trachomatis in synovial fluids from patients with reactive arthritis using the polymerase chain reaction and antigen detection methods. Br J Rheumatol 1992;31:31-40
12. Sieper J, Braun J, Wu P, Hauer R, Laitko S. The possible role of Shigella in sporadic enteric reactive arthritis. Br J Rheumatol 1993;32:582-85
13. Fendler C, Wu P, Eggens U, et al. Longitudinal investigation of bacterium-specific synovial lymphocyte proliferation in reactive arthritis and lyme arthritis. Br J Rheumatol 1998;37: 784-88
14. Granfors K, Merilahti-Palo R, Luukkainen R, et al. Persistence of Yersinia antigens in peripheral blood cells from patients with Yersinia enterocolitica O:3 infection with or without reactive arthritis. Arthritis Rheum 1998;41:855-62
15. Gupta RC, Badhwar AK, Bisno AL, Berrios X. Detection of C-reactive protein, streptolysin O, and anti-streptolysin O antibodies in immune complexes isolated from sera of patients with acute rheumatic fever. J Immunol 1975;137:2173-79
16. Storellman GH. Rheumatic fever and streptococcal infection. New York, NY, Grune and Stratton. 1975
17. Svartman M, Potter EV, Poon-King T, Earle DP. Immunoglobulins and complement components in synovial fluid of patients with acute rheumatic fever. J Clin Invest 1975;56: 111-17
18. Ziff M, Cohen BS. Atherogenicity of streptococcal cell walls. Adv Inflam Res 1985;9:1-17
19. Bronze MS, Beachey EH, Dale JB. Protective and heart-crossreactive epitopes located within the NH2 terminus of type 19 streptococcal M protein. J Exp Med 1988;167: 1849-59
20. Lancefield RC. New approaches for the laboratory recognition of M types of group A streptococci. J Exo Med 1971;134:1298-315
21. Baird RW, Bronze MS, Kraus W, Hill HR, Veasy LG, Dale JB. Epitopes of group A streptococcal M protein shared with antigens of articular cartilage and synovium. J Rheumatol 1991;146:3132-37
22. Greenblatt JJ, Hunter N, Schwab JH. Antibody response to streptococcal cell wall antigens associated with experimental arthritis in rats. Clin Exp Immunol 1980;42:450-57
23. Ferriero P; Jones Criteria Working Group. Proceedings of the Jones Criteria Workshop. Circulation 2002;106:2521-23
24. Chagani HS, Aziz K. Clinical profile of acute rheumatic fever in Pakistan. Cardiol Young
25. Report of a WHO Expert Consultation. World Health Organization, Geneva, 2004; Technical Report Series No. 923.
26. Pereira BA, da Silva NA, Andrade LE, Lima FS, Gurian FC, de Almeida Netto JC. Jones criteria and underdiagnosis of rheumatic fever. Indian J Pediatr 2007;74:117-21.
27. Shulman ST, Ayoub EM. Poststreptococcal reactive arthritis. Curr Opin Rheumatol 2002;14:562-65.
28. Sakurai S, Kosihara T, Otake M, Saito T, Machida H, Takagi T. Clinical features of patients with osteoarthritic knees followed by development of rheumatoid arthritis. Arthritis Rheum 2000; 40: 685-92. Abstract.
29. Salton MRJ, Horn RW. Studies on the bacterial cell wall I. Methods of preparation and some properties of cell walls. Biochim Biophys Acta 1951, 6: 177-97. Abstract.
30. Granfors K, Jalkanen S, Von Essen R, et al. Yersinia antigens in synovial fluid cell from patients with reactive arthritis. N Engl J Med 1989;320:216-21.
31. Stahlberg TH, Granfors K, Toivanen A. Immunoblot analysis of human IgM, IgG and IgA responses to plasmid-encoded antigens of Yersinia enterocolitica serovar O3. J Med Microbiol 1987;24:157-63.
32. Hawkes R, Niday E and Gordon J. A dot-immunobinding assay for monoclonal and other antibodies. Anal Biochem 1982; 119: 142-47.
33. Bisno AL. Group A streptococcal infections and acute rheumatic fever. New England J Med 1991; 325: 783-93.
34. Stollerman GH. Rheumatic fever. Lancet 1997;349:935-42
35. Carapetis JR, Currie BJ, Good M. Towards understanding the pathogenesis of rheumatic fever. Scandinavian J Rheumatol 1996;25: 127-31.
36. Stollerman GH. Rheumatic fever in the 21st century. Clin Infect Dis 2001;33:806-14.
37. Stollerman GH. Current issues in the prevention of rheumatic fever. Minerva Med 2002;93:371-87.
38. Nesher G, Moore TL, Grisanti MW, el-Najdawi E, Osborn TG. Correlation of antiperinuclear factor with antibodies to streptococcal cell-wall peptidoglycan-polysaccharide polymers and rheumatoid factor. Clin Exp Rheumatol 1991;9:611-15.
39. Appel H, Mertz A, Distler A, Sieper J, Braun J. The 19 kDa protein of Yersinia enterocolitica O:3 is recognized on the cellular and humoral level by patients with Yersinia induced reactive arthritis. J Rheumatol 1999;26:1964-71.
40. Schnarr S, Putschky N, Jendro MC, et al. Chlamydia and Borrelia DNA in synovial fluid of patients with early undifferentiated oligoarthritis: results of a prospective study. Arthritis Rheum 2001;4:679-85.
41. Thiel A, Wu P, Lauster R, Braun J, Radbruch A, Sieper J. Analysis of the antigen-specific T cell response in reactive arthritis by flow cytometry. Arthritis Rheum 2000;43:2834-42.
42. Heymer B, Schleifer KH, Read S, Zabriskie JB, Krause RM. Detection of antibodies to bacterial cell wall peptidoglycan in human sera. J Immunol 1976;117:23-26.
43. Johnson PM, Phua KK, Perkins HR, Hart CA, Bucknall RC. Antibody to streptococcal cell wall peptidoglycan-polysaccharide polymers in seropositive and seronegative rheumatic disease. Clin Exp Immunol 1984;55:115-24.