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Immunoglobulin-coated bacteria on the tonsillar surface during infectious mononucleosis

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Abstract

Sequential bacterial samples were obtained from the tonsillar surface of 19 consecutive patients (12 females, seven males; mean age 16.1 years, range four to 24 years) suffering from infectious mononucleosis with membranous tonsillitis. The specimens were examined with respect to aerobes (culture on blood and chocolate agar plates) and proportions of bacteria coated with immunoglobulins (secretory IgA, IgG, IgM) by using an immunofluorescence assay. In the early stage of the membranous tonsillitis phase, attachment of secretory IgA (SIgA) and IgG to the bacteria was greatly suppressed, as compared with healthy controls. Coating with IgM was evident only late in the membranous tonsillitis phase but was contracted and still evident even after the clinical throat symptoms had abated. The findings suggest that the B-lymphotropic Epstein-Barr virus, causative agent of infectious mononucleosis, exerts a transient suppression of immunoglobulin-coating of bacteria harboured on the tonsillar surfaces, with consequent abundant bacterial attachment to the epithelial cells and massive bacterial colonization on the palatine tonsils.

Key words: Epstein-Barr virus; Immunoglobulins; Mucosal immunity

Introduction

One of the most striking symptoms during the course of acute infectious mononucleosis (IM) is the involvement of the palatine tonsils, which become covered by a membranous exudate composed of many different species of bacteria (Stenfors and Räisänen, 1993). Primary infection with Epstein-Barr virus (EBV) is usually asymptomatic, but may result in acute IM in young adults and sometimes even during childhood (Henle and Henle, 1970; Epstein and Achong, 1977). B-lymphocytes and oropharyngeal epithelial cells are the chief target cells of EBV infection (Schuster and Kreth, 1992). Antigen-stimulated B lymphocytes can migrate to secretory sites via lymph and peripheral blood and there form mature immunoglobulin-secreting plasma cells. About two per cent of the mononuclear cell population of the tonsils constitute immunoglobulin-secreting cells (Nadal *et al.*, 1992).

Hoagland (1960) has defined the clinical and laboratory criteria for IM, which include fever, tonsillopharyngitis, lymphadenopathy, splenohepatomegaly, absolute lymphocytosis with more than 10 per cent atypical Downey-McKinley lymphocytes, and detectable heterophile antibodies in serum according to Paul and Bunnell (1932).

The purpose of the present study was to evaluate whether or not the B-lymphotropic Epstein-Barr virus could affect immunoglobulin-coating of the bacteria present on the palatine tonsils. In the present study, a sequential analysis of the micro-organisms covering the tonsillar surfaces during a current IM infection, with respect to attachment of immunoglobulins (SIgA, IgG, IgM) to the bacterial wall, was performed.

Patients and methods

Nineteen patients (12 females, seven males; mean age 16.1 years, standard deviation 4.2 years, range four to 24 years), were referred to our Outpatient Department, suffering from throat symptoms of one to 14 days' duration. All patients fulfilled the criteria of IM (Hoagland, 1960) with detectable heterophile antibodies in the serum and a positive rapid test for IM antibodies (Monosticon, Turnout, Belgium). None of the patients exhibited serum immunoglobulin concentrations below normal at the start of the study. Five patients had elevated serum IgM concentrations. Serum immunoglobulins were measured by an immunoturbidimetric assay.

Throat swab specimens were obtained from both palatine tonsils by rotating a cotton wool tipped wooden swab, which had been dipped in physiological saline, twice on the tonsillar surface. Specimens were obtained when the patient first arrived at the Department (Day 1), and on Days 5, 10 and 30. Each

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swab was placed in a tube containing 2 ml physiological saline and then immediately processed as follows:

(1) Samples were smeared over blood-agar and chocolate-agar plates for standard aerobic culturing according to routine laboratory procedure (kept in five per cent CO_2 at 37°C for 48 hours).

(2) Samples were smeared over clean glass slides, allowed to dry, and fixed with 70 per cent alcohol solution. The slides were stored at −80°C until immunofluorescence processing. After thawing, three hydrophobic rings were drawn on each dried glass slide. Within each ring, two drops of fluorescein-conjugated goat anti-human IgG (1201-0081, Cappel, Organon Teknika Corporation, West Chester, USA)-, flurorescein conjugated goat anti-human secretory IgA (SIgA; 1301-0011, Cappel)-, or fluorescein-conjugated goat anti-human IgM-serum (F-5384, Sigma Chemical Co., St Louis, USA) were added, respectively. Counterstaining was performed with 0.25 per cent Evans blue. All samples were processed simultaneously, using the same reagent and its various dilutions. The specimens were examined under a Leitz fluorescence microscope. One hundred bacteria from four different areas in each ring were evaluated. Immunoglobulin-coated bacteria were stained green, whereas non-coated bacteria stained red. The method has been described in detail previously (Stenfors and Räisänen, 1991).

Prior to the immunofluorescence studies, the antisera were titrated according to the principles promulgated by the U.S. Department of Health, Education and Welfare (1972).

Samples obtained and processed similarly were collected from 19 healthy, age- and sex-matched volunteers. These subjects had not suffered recently from any respiratory tract infection and no antibiotics had been prescribed within three months of the sampling.

For statistical analyses, the Wilcoxon matched-pairs sign rank test and McNemar's test were used. Informed consent was obtained from the parents prior to the study. The study was approved by the Medical Ethics Committee of our hospital.

Results

The results are summarized in Table I and Figure 1.

Controls

In the healthy controls (n = 19), 86 per cent (median value) of the bacteria harvested from the tonsillar surfaces showed positive reactivity when treated with anti-human SIgA serum (Figure 2), and 94 per cent (median value) evidenced positive

TABLE I

PROPORTIONS (%) AND MEDIAN VALUES OF TONSILLAR SURFACE BACTERIA SHOWING REACTIVITY WITH ANTI-SIGA, ANTI-IGG AND ANTI-IGM SERUM IN HEALTHY CONTROLS (N = 19) AND DURING THE COURSE OF INFECTIOUS MONONUCLEOSIS (N = 19) ON DAY 1, 5, 10 AND 30.

				Infectious mononucleosis											
	Controls			Day 1			Day 5			Day 10			Day 30		
Pair no.	SIgA	IgG	IgM	SIgA	IgG	IgM	SIgA	IgG	IgM	SIgA	IgG	IgM	SIgA	IgG	IgM
1	90	90	6	74	100	0	16	20	0	68	79	0	76	65	0
2	88	94	0	52	88	0	14	26	0	90	94	68	64	94	10
3	100	96	0	90	100	64	80	80	60	62	58	33	72	98	0
4	86	96	0	100	100	100	82	80	60	88	86	30	86	60	0
5	86	94	4	88	92	88	78	84	40	60	68	40	76	90	20
6	82	100	0	8	18	0	10	14	6	92	90	20	78	88	20
7	48	92	0	10	0	26	10	14	20	68	74	20	82	64	20
8	100	100	0	0	0	62	62	94	12	66	90	0	82	78	0
9	90	100	0	24	14	8	30	34	20	78	80	18	70	92	10
10	80	92	0	14	0	0	24	44	0	60	60	0	80	86	0
11	96	80	0	22	92	8	44	84	12	56	64	8	76	98	0
12	100	100	0	100	88	86	90	88	10	90	86	8	92	68	9
13	72	86	0	6	8	0	14	28	30	58	80	26	66	88	20
14	64	82	0	0	0	0	28	38	20	66	78	20	62	96	20
15	48	62	0	0	12	0	12	76	56	86	70	38	92	74	50
16	74	84	0	0	12	0	26	0	0	46	54	40	88	90	40
17	92	94	0	32	22	0	34	34	10	86	88	36	90	88	20
18	84	96	0	0	0	0	28	46	30	58	50	30	88	100	80
19	78	86	0	0	0	0	24	30	36	56	64	40	74	90	0
SigA		86			14 $p<0.0005$			28 $p<0.0005$			66 $p<0.025$			78 p = NS	
IgG		94			14 $p<0.005$			38 $p<0.0005$			78 $p<0.0005$			88 p = NS	
IgM*		0 (2/19)			0 (8/19) p = NS			20 (15/19) $p<0.01$			26 (16/19) $p<0.01$			10 (12/19) $p<0.01$	

*Number of individuals with IgM-coated bacteria are added.

Differences in SIgA- and IgG-coated bacteria between the controls and IM-groups were calculated using the Wilcoxon matched-pairs sign rank test and in a number of individuals with IgM-coated bacteria using McNemar's test.

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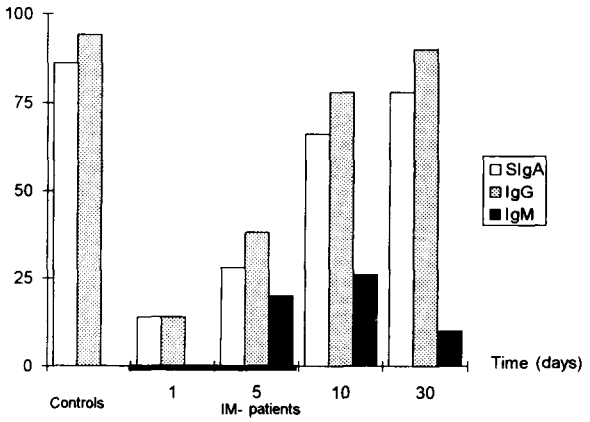

FIG. 1

Proportions of bacteria (median values) obtained from the tonsillar surface of healthy controls (n = 19) and during the course of infectious mononucleosis (n = 19), showing reactivity with anti-SIgA, anti-IgG and anti-IgM serum. The membranous tonsillitis phase is indicated.

reactivity to anti-human IgG serum. Only two samples displayed bacteria with some positive reactivity when treated with anti-human IgM serum. Aerobic culturing revealed growth of bacterial non-pathogens on the tonsillar surface, α-haemolytic streptococci, *Neisseria* spp., *Corynebacteria* spp. and various spirochetae. Two controls, however, showed growth of non-typable *Haemophilus influenzae*.

Patients

All patients exhibited a typical membranous tonsillitis on Day 1 or Day 5. IgA- and IgG-coating of the bacteria was significantly reduced during the membranous tonsillitis phase of the infectious mononucleosis infection (p<0.005). Seven samples failed to show any IgG-coated bacteria and six samples no SIgA-coated bacteria. These non-coated IgG and SIgA bacterial samples were all obtained on Day 1 or Day 5. In addition, if SIgA- or IgG-coated bacteria were present on Day 1 or Day 5, the immunoglobulin-coating was significantly reduced compared to the healthy controls. On Day 30, the SIgA- and IgG-coating of the bacteria was normal and no significant difference compared to the controls could be found. Seventeen of the IM individuals had IgM-coated bacteria. This too was a highly significant difference (p<0.01). The samples with significant differences in IgM-coating were found on Days 5, 10 and 30, i.e. in the late phase of the membranous tonsillitis stage and even after all throat symptoms had disappeared. The differences regarding IgM-coating of the tonsillar surface bacteria between the IM patients and the controls are calculated using McNemar's test because only two out of 19 controls harboured bacteria. Among the IM-patients, seven out of 19 on Day 1, 15 out of 19 on Day 5, 16 out of 19 on Day 10 and 12 out of 19 on Day 30 hosted IgM-coated bacteria on the palatine tonsils.

In the IM group, seven patients harboured β-haemolytic streptococci, four with type C, the remainder with type A, type G or type F. Generally speaking, aerobic culturing revealed massive growth of the flora normally harboured on the tonsillar surfaces, viz. *Streptococcus viridans*, *Neisseria* spp., *Corynebacteria* spp., and various spirochaetae.

Discussion

The present study showed that the bacteria located on the tonsillar surfaces of young persons (control group) in the vast majority of cases are coated with SIgA and IgG antibodies but not with IgM. In a previous study (Stenfors and Räisänen, 1993), we showed that the fur forming the membranous tonsillitis during an IM infection consists mainly of a bacterial mass intermingled with inflammatory cells and cellular detritus. The abundant attachment of bacteria to the epithelial cells was typical. The present study showed that in the early phase of the membranous tonsillitis stage, the immunoglobulin protection of the tonsillar surfaces was greatly suppressed and the bacteria were insufficiently coated with SIgA and IgG antibodies. In the late phase of the membranous stage of the tonsillitis, immunoglobulin-coating was replaced by IgM. It is conceivable that the increased attachment of bacteria to the epithelial cells was due to a transient suppression in coating of the bacteria with SIgA. One of the main functions of SIgA is to hinder bacterial attachment to epithelial surfaces (Williams and Gibbons, 1972; Kurono *et al.*, 1991).

The abundant bacterial attachment could, of course, pave the way for the massive bacterial colonization of the tonsillar surfaces, further maintained by inadequate IgG-coating of the bacteria. It

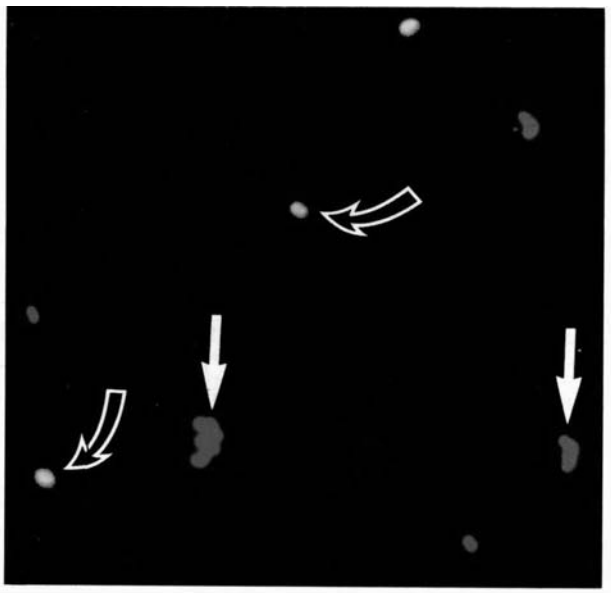

FIG. 2

Photograph of bacterial sample obtained from the tonsillar surface, treated with fluorescein-labelled anti-human SIgA serum, counterstained with 0.25% Evans blue. Bacteria showing positive reactivity are stained green (open arrows) and those without reactivity are stained red (filled arrows) (× 3420).

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is well known that IgG is of importance for bacteriolysis, but can also provoke opsonization of the microorganisms (McNabb and Tomasi, 1981).

During healthy conditions only minimal IgM-coating of the bacteria harboured on mucosal membranes takes place (control group). IgM-coating of the bacteria on the tonsillar surfaces during IM infection coincided unambiguously with the repulsive process of the fur causing the membranous tonsillitis. Furthermore, a significant number of IgM-coated bacteria were detected on the tonsillar surfaces, even on Day 30 when the patients were free from throat symptoms and the palatine tonsils looked quite normal. This finding also suggests that IgM antibodies take part in the immune protection of mucosal membranes as previously suggested (Brandtzaeg, 1992).

None of the IM patients had reduced serum immunoglobulin levels. Five of the patients had elevated serum IgM concentration on Day 1. In 1919 Besredka proposed that there exists a protective local immune system that seems to function independently of systemic immunity. The molecular basis for local immunity was established when Tomasi *et al.* (1965) confirmed that external secretions contained a unique immunoglobulin, subsequently called secretory IgA (SIgA). The present study could unambiguously confirm the ideas put forward by Besredka.

The main target cells for the EBV attack are oropharyngeal epithelial cells and B-lymphocytes (Schuster and Kreth, 1992). When antigen is presented for the B-lymphocytes, these cells can move via the efferent lymphatics and reach the systemic circulation through the thoracic duct. Circulating B-lymphocytes then enter distant mucosal tissues, where the cells clonically expand and mature into immunoglobulin-secreting plasma cells. It cannot be excluded that this event is highly disturbed during an EBV infection.

Besides being the causative agent for IM infection, EBV is also implicated in the pathogenesis of different malignant tumours such as Burkitt's lymphoma, nasopharyngeal carcinoma, Hodgkin's disease and some T-cell lymphomas (Schuster and Kreth, 1992). These clinically important coincidences, make EBV studies extremely important.

References

Besredka, A. (1919) De la vaccination contre les états typhoides parla voie buccale. *Annales de l'Institut Pasteur* **33:** 882–903.

Brandtzaeg, P. (1992) Humoral immune response patterns of human mucosae: Induction and relation to bacterial respiratory tract infections. *Journal of Infectious Diseases* **165 (suppl 1):** 167–176.

Epstein, M. A., Achong, B. A. (1977) Recent progress in Epstein-Barr virus research. *Annual Review of Microbiology* **31:** 421–445.

Henle, G., Henle, W. (1970) Observations on childhood infections with Epstein-Barr virus. *Journal of Infectious Diseases* **121:** 303–310.

Hoagland, R. J. (1960) The clinical manifestations of infectious mononucleosis. A report of two hundred cases. *American Journal of Medical Sciences* **240:** 55–62.

Kurono, Y., Shimamura, K., Shigemi, H., Mogi, G. (1991) Inhibition of bacterial adherence by nasopharyngeal secretions. *Annals of Otology, Rhinology and Laryngology* **100:** 155–158.

Laboratory outline for training course in principles and bacterial applications of fluorescent antibody techniques. (1978) U.S. Department of Health, Education, and Welfare, Public Health Service, Centers for Disease Control, Atlanta, Georgia 3033, USA., pp 53–83.

McNabb, P. C., Tomasi, T. B. (1981) Host defence mechanisms at mucosal surfaces. *Annual Review of Microbiology* **35:** 477–496.

Nadal, D., Soh, N., Schläpfer, E. (1992) Distribution characteristics of immunoglobulin-secreting cells in adenoids. Relation to age and disease. *International Journal of Pediatric Otorhinolaryngology* **24:** 121–130.

Paul, J. R., Bunnell, W. W. (1932) The presence of heterophile antibodies in infectious mononucleosis. *American Journal of Medical Sciences* **183:** 91–104.

Schuster, V., Kreth, H. W. (1992) Epstein-Barr virus infection and associated diseases in children: Pathogenesis, epidemiology and clinical aspects. *European Journal of Pediatrics* **151:** 718–725.

Stenfors, L.-E., Räisänen, S. (1991) Secretory IgA- and IgG-coated bacteria in the nasopharynx of children. *Acta Otolaryngologica* **111:** 1139–1145.

Stenfors, L. -E., Räisänen, S. (1993) The membranous tonsillitis during infectious mononucleosis is nevertheless of bacterial origin. *International Journal of Pediatric Otorhinolaryngology* **26:** 149–155.

Tomasi, T. B., Tan, E. M., Salomon, A., Prendergast, R. A. (1965) Characteristics of an immune system common to certain external secretions. *Journal of Experimental Medicine* **121:** 101–124.

Williams, R. C., Gibbons, R. J. (1972) Inhibition of bacterial adherence by secretory immunoglobulin A: a mechanism of antigen disposal. *Science* **177:** 697–699.

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