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The role of serum factors and influence of intravenous immunoglobulins on phagocytosis of campylobacter species

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Rapid expansion of our knowledge on the role of Campylobacter species in gastrointestinal diseases was made recently. C. jejuni (and less frequently C. coli) is a common cause of self-limiting diarrhoea in normal subjects [1, 13] and of chronic diarrhoea in patients with hypogammaglobulinemia. C. fetus, although more often causing septicaemia in immunocompromised hosts [11], chronic alcoholics and other patients with lowered resistance to infections [2, 14], may also be found in stools. C. pylori has been isolated from the antral musosa of the stomach in patients with gastritis and duodenal ulcers and may be involved in their aetiology [8].

Our interest in the gut flora of hypogammaglobulinemic patients prompted us to study the opsonisation of Campylobacter species for neutrophil phagocytosis, with the aim to find whether antibody is critically required. The influence of immunoglobulin substitution therapy in antibody deficient children on opsonization of Campylobacter bacilli was also studied.

1. Bacterial species. The following Campylobacter species were used: C. jejuni strain 81116 (Dr Newell, Southampton) and strain 53729/80 (Northwick Park Hospital), C. coli, NCTC 11353, C. fetus, NCTC 10842 and various strains of C. pylori isolated from gastric antral biopsy tissue of patients with duodenal ulcers.

2. Sera. Fresh serum samples were obtained from laboratory workers and blood donors in Spain, England and Poland, and frozen at −70 °C within 30 min. Serum samples were also obtained from a patient with homozygous C2 deficiency and from 11 children with hypogammaglobulinemia during substitution therapy with IVIG in two doses — 500 mg/kg b.w. (group A) and 150 mg/kg b.w. (group B).

3. Antibody and Chemiluminescence Assays. Campylobacter species at 5×10^9 organisms/ml in PBS, harvested and washed, were diluted 1 : 750 in PBS and killed at 60 °C for 1 hr. Microwell plates were coated with 100 μl of the bacterial suspension (2 hr 37 °C) and prepared for indirect

ELISA test with class-specific peroxidase conjugated anti human Ig (Sigma). The bunding of bacteria to neutrophils (opsonisation) was measured by chemiluminescence with 25 μl of the tested sera (7a).

Antibody assays. A total of 33 subjects were tested for antibody to the various Campylobacter species: 5 Spanish, 5 British, 15 Polish and 1 Argentinian. The data suggest that there are geographical differences in the exposure to these organism. The only one argentinian subject had a high titre of antibodies to C. pylori and he subsequently suffered from a duodenal ulcer.

Opsonisation. The chemiluminescence released from normal neutrophils with the Campylobacter species associated with large bowel disease showed a similar pattern: very little reaction with heat-inactivated (decomplemented) serum, but a marked rise in chemiluminescence with fresh serum. The fresh serum used for C. jejuni was known to contain antibody by ELISA, implying that antibody and complement were necessary for significant opsonisation in this system. Low luminescence occurred with C2-deficient serum which also contained some antibody, suggesting that either antibody alone or activation through the alternative pathway can cause binding, albeit inefficiently. Pooled IgG containing measurable antibody to C. jejuni gave a low level of chemiluminescence, indicating that some IgG antibodies are effective opsonins in their own right: hypogammaglobulinemic serums, as a source of complement, enhanced the chemiluminescence. In contrast, C. pylori induced the chemiluminescence in the absence of opsonins, implying direct binding of the organism to receptors on the neutrophil surface. Electron microscopy of neutrophils at the and of this assay showed many bacteria within phagocytic vacuoles. The opsonisation of C. pylori was enhanced by the presence of fresh serum containing antibody and complement, but was partially inhibited by C2-deficient serum and by heated serum. Opsonisation of bacteria C. jejuni 81116 was tested by chemiluminescence in the presence of serum of children with antibody deficiency syndrome before and 24 hrs after infusion with IVIG. The opsonizing capacity of sera raised depending on a dose of infused IgG.

Discussion

Campylobacter bacteria have been on the mucosal surfaces of the stomach, small and large intestine, and in the latter are clearly a cause of mucosal inflammation and diarrhoea [12]. Bacteria are rarely seen penetrating the mucosal surface, although superficial ulceration in the large bowel may occur in immunodeficient patients with chronic campylobacter enteritis [7]. As with many other intestinal organisms, s-IgA antibody may have an important protective role [4], although there is no evidence that selective IgA deficiency predisposes to Campylobacter infection. However,

patients who lack all clases of antibodies are prone to C. jejuni enteritis, suggesting that IgG and/or IgM antibodies may be more important. Patients with hypogammaglobulinemia commonly develop achlorhydria, which is thought to be predisposing factor for their high incidence of gastric carcinoma [5]. C. pylori has been grown from the gastric juice and antral mucosa in 2 out of 3 hypogammaglobulinemic patients with achlorhydria so far tested in Northwick Park Hospital. This supports a thesis for the role of antibody in controlling the growth of C. pylori, particularly as achlorhydric patients with pernicious anaemia are rarely colonized [9]. The role of neutrophils and macrophages in preventing mucosal colonisation by bacteria is not clear. However, neutrophils are likely to be attracted to small tears in the mucosa, in the same way as they are to any other injured tissue. This may not be advantageous in the mucosa since phagocytosis of bacteria by neutrophils may stimulate the production of inflammatory mediators and cause ulceration. If this is true, then there are likely to be factors (e.g. certain classes and subclasses of antibodies, esp. s-IgA) which inhibit the phagocytosis of mucosal organisms, while at the some time inhibiting their growth. We have shown that three Campylobacter species associated with large bowel infection are opsonised for neutrophil phagocytosis by a combination of antibody and complement in serum. However, it is unclear whether there is a sufficient concentration of IgG antibody or complement in mucosal secretions to mediate this phenomenon in vitro. Furthermore, the possibility that s-IgA or IgG2 antibodies in secretions block opsonisation should now be explored.

The finding that C. pylori binds spontaneously to neutrophils is important because it shows that the organism is capable of activating these cells in the absence of antibody or complement. This might explain the severe gastritis in patients with hypogammaglobulinemia, which unlike the gastritis in most cases of classical pernicious anaemia involves the antrum (Webster 1986). Although we have been unable to demonstrate serum antibodies which inhibit the opsonisation of C. pylori a study of the inhibiting properties of specific s-IgA antibodies in secretions now seems appropriate. There may also be other inhibiting factors in serum which operate in the absence of complement, as suggest by the inhibition of chemiluminescence with C2 eficient and heated serum. Further work in this area may throw light on the aetiology of duodenal ulcer and lead to a new approach to therapy. Infections with Camp. bacteria in children with primary antibody deficiency are quite common and may be the cause of dramatic complications [10]. The concentration of antibodies anti-Campylobacter in both groups of children after IVIG infusion rised substantially and attained the level of the control group. Similarly, we noted the increase of opsonising capacity for C. jejuni.

References

1. Blaser MJ, Reller LB: Campylobacter enteritis. N Engl J Med 305: 1444, 1981
2. Guerrant RL, Lahita RG, Winn WC, Roberts RB: Campylobacteriosis in man: Pathogenic mechanisms and review of 91 bloodstream infections. Am J Med 65: 584, 1978
3. Hanson LA, Ahlstedt S, Andersson B, Carlsson B, Dahlgren U, Lidin-Jonson G, Mattsby-Bultzer I, Svanberg-Eden C: The biologic properties of secretory IgA. J Leukoc Biol 28: 1s, 1980
4. Johnson RJ, Nolan C, Wang SP, Shelton WR, Shelton SP, Blaser MJ: Persistent Campylobacter jejuni infection in an immunocompromised patient. Ann Int Med 100: 832, 1984
5. Kinlen LJ, Webster ADB, Bird AG, Haile R, Peto J, Soothill JF, Thompson RA: Prospective study of cancer in patients with hypogammaglobulinemia. Lancet 1: 263, 1985
6. Lever AML, Dolby JM, Webster ADB, Price AB: Chronic Campylobacter colitis and uveitis in patients with hypogammaglobulinaemia. Brit Med J 288: 531, 1984
7. Lever AML, Gross J, Webster ADB: Serum factors for opsonisation of non-typical Haemophilus influenzae. J Med Microbiol 20: 33, 1985
8. Marshall BJ, Armstrong JA, McGehie DB, Glancy RJ: Attempt to fulfil Koch's postulates for pyloric campylobacter. Med J Australia 142: 436, 1985
9. O'Connor HJ, Axon ATR, Dixon MF: Campylobacter-like organisms unusual in type A (pernicious anaemia) gastritis. Lancet 2: 1091, 1984
10. Pönkä A, Tilvis R, Kosunen TU: Prolonged Campylobacter gastroenteritis in patient with hypogammaglobulinemia. Acta Med Scand 213: 159, 1983
11. Righter J, Woodrow AW, Hart GD, McNeely DJ: Relapsing septicaemia caused by Campylobacter fetus subsp. fetus. Canad Med Assoc J 128: 686, 1983
12. Riley LW, Finch MJ: Results of the first year of national surveillance of Campylobacter infections in the United States. J Inf Dis 151: 956, 1985
13. Skirrow MB: Campylobacter enteritis. Brit Med J 2: 9, 1977
14. Targan SR, Chow AW, Guze LB: Spontaneous peritonitis in cirrhosis due to Campylobacter fetus. Gastroenterol 11: 311, 1976
15. Webster ADB: Immunodeficiency and gastrointestinal disease. In: Clinical Immunology of the liver and gastrointestinal tract, ed Triger DR, John Wright and Sons Ltd, 1986