

The intestinal immune system

WILLIAM F DOE

(This article is one of a series linked with the Festschrift for Christopher Booth. See Gut Festschrift 1989; 30.)

The concept of localised intestinal immunity has its origins in Besredka's observation that oral immunisation using killed salmonella organisms provided solid protection against dysenteric infection irrespective of the titres of serum antibody.¹ But the clinical benefits were variable and short lived and interest lapsed. Little further progress was made until Heremans' discovery of IgA and the marked predominance of IgA-containing plasma cells in normal intestinal mucosa.² The differences between the antigenic determinants of serum and secretory IgA led to the identification of an additional polypeptide chain, the secretory component (SC), which is essential to the secretion and function of IgA antibody at mucosal surfaces.³

The importance of the mucosal immune system to the pathogenesis of intestinal disease was swiftly recognised by Chris Booth. When I arrived as a houseman at Hammersmith in 1969, several pioneering immunological studies of coeliac disease had been published. The clinical research ethos at Hammersmith stimulated the study of human disease not only by applying basic scientific knowledge and techniques but also by recognising that careful study of human disease can also provide opportunities for advancing our understanding of human biology. I recall the outpatient clinic in 1969 when Chris handed me the referral letter for a new patient he had not seen: 'Fascinating, sounds like alpha chain disease.'

Several months later Heremans came to Hammersmith to deliver a series of outstanding lectures on the secretory immune system. Grand rounds that week included the presentation of what was to be one of the earliest published cases of alpha chain disease.⁴

In the decades since these early discoveries, major advances in understanding the physiology of the intestinal immune response and the advent of molecular biology have helped to elucidate the induction and regulation of the secretory antibody and cellular responses at mucosal surfaces.

Gut associated lymphoid tissue (GALT)

The exposed surface of the intestinal mucosa is under constant challenge by ingested foreign antigens in micro-organisms, products of food digestion and drugs. It is therefore not surprising that the intestines

contain the largest accumulation of lymphoid tissues in the body⁵ in the form of lymphoid aggregates in Peyer's patches and in the lamina propria (solitary lymphoid nodules) and as the scattered lymphocyte populations found in the epithelium and in the lamina propria.

Although nascent Peyer's patches are evident in the newborn, the epithelium and lamina propria are devoid of mononuclear cells. T lymphocytes migrating from the thymus rapidly populate the thymus – dependent areas of Peyer's patches and the epithelium but exposure to micro-organisms in the normal environment is necessary to develop the B cell population and their germinal follicles as shown by experiments conducted in germ free animals.⁶

Induction of the secretory immune response

Non-immune and immune mechanisms protect the privileged environment of the lamina propria from challenge by foreign antigens. Gastric acidity, digestive proteases in the gastrointestinal tract, intestinal mobility, the commensal microflora and the mucous coat or glycocalyx comprise some of the non-specific protective barriers. The immune mechanisms may operate within the lumen of the gut, at the mucosal surface or within the lamina propria. The inter-epithelial lymphocyte (IEL) population are predominantly suppressor lymphocytes (Ts) in contrast with the lamina propria and show evidence of activation whereas most of the lamina propria lymphocytes belong to the helper-inducer subset (Th). Class II MHC determinants which represent a restriction element in T cell dependent immune responses are expressed on normal small intestinal epithelial cells but not colon epithelial cells unless the colon is inflamed. Expression of class II antigens is modulated by lymphokine products of activated T cells especially interferon γ (IFN γ). Bland and Warren⁷ reported that MHC class II positive villous epithelial cells can present soluble antigen to primed T cells leading to antigen specific suppression. Although human colonic epithelial cells have been reported to act as stimulators in autologous and allogeneic responses⁸ others have been unable to stimulate T cells in an allogeneic system using an MHC class II positive colon cancer cell line.⁹

Whether IEL require a second signal from accessory cells in the lamina propria remains to be answered.

Most studies of isolated GALT cells have been performed using Peyer's patch cells. Whether these reflect the function of all organised GALT is unknown. The dome of Peyer's patches is covered by a unique epithelium comprising cuboidal epithelial cells which express class II MHC antigens, very few goblet cells and specialised antigen-sampling cells called M (for microfold) cells.¹⁰ M cells pinocytose soluble antigens such as ferritin and horse-radish peroxidase or phagocytose particulate foreign antigens including viruses and whole bacteria, and transport them intact across the epithelium to the underlying lymphoreticular cells in the dome.¹¹

Macrophages and dendritic cells are present in the dome region, and MHC class II positive cells displaying dendritic cell morphology are also found in the T and B cell zones. Dendritic cells found in cell suspensions of Peyer's patches are fully able to present antigen *in vitro*¹² and those present in the lamina propria of the small and large intestine in mouse and man are also competent to present antigens. Whether Peyer's patch macrophages also function as antigen presenting cells, however, is uncertain, as lamina propria macrophages suppress antigen presentation by dendritic cells.¹³

Peyer's patches contain a higher proportion of B cells than peripheral nodes. While IgM-bearing B cells predominate, there is significant enrichment for B cells displaying surface IgA and committed to IgA synthesis consistent with the role of GALT as a major site for the induction of IgA responses. Peyer's patches are also greatly enriched for T cells of the helper-inducer subset (Th) although suppressor-cytotoxic T cells (Ts) and the regulatory contra-suppressor T cells (Tcs) which appear to potentiate immune responses to orally presented antigen, are also found. The high degree of preferential localisation of B cells and IgA specific Th cells in Peyer's patches is probably determined by the specificity of binding of these lymphocytes to the high endothelial venule receptors present in postcapillary venules in Peyer's patches¹⁴ which determine their representation in Peyer's patches and ultimately regulate the nature of the immune response generated.

Yet induction of mucosal immunity and the delivery of the immune response is not entirely localised to the intestine. After antigen exposure, Th cells and B cells committed to specific IgA synthesis are rapidly generated in Peyer's patches and travel *via* the mesenteric node into the thoracic duct to enter the circulation before selectively migrating to mucosal surfaces. Whether this inability to mount a completely localised immune response to antigen encountered at

a mucosal surface relates to separation of the T and B cell zones in Peyer's patches from antigen presenting cells is not known. Mestecky and McGhee¹⁵ have speculated that antigen presentation to lymphocytes does occur in GALT resulting in an 'initial induction' of immune responses and that the 'terminal inductive stimuli' may occur at distant mucosal sites after these committed lymphocytes have migrated there.

The migratory characteristics of IgA-committed lymphoblasts and memory cells are quite distinct. Unlike memory lymphocytes, IgA-containing lymphoblasts do not recirculate but 'home' to the organ of antigenic stimulation, secrete antibody, remain associated with the mucosal target tissue and probably die within a few days.¹⁶ In rats primed and challenged by intraintestinal injection of cholera toxin, specific antibody – containing lymphoblasts were found three to five days later in the thoracic duct lymph from where they entered the circulation and then populated distant mucosal sites. The number of lymphoblasts in the thoracic duct greatly diminished after five days but the number of small lymphocytes capable of transferring specific immunity to naive animals (memory cells) peaked after two weeks and persisted. The memory cells, which will produce T- and B-lymphoblasts when stimulated by specific antigen, enter the circulation from the lymph, 'home' to distant mucosal sites and then re-enter the lymph to recirculate.¹⁷ Peripheral lymphoid tissues were not examined in these experiments but other studies suggest that memory lymphocytes from the thoracic duct also 'home' preferentially to mucosal lymphoid tissue.

Lymphocyte 'homing' is not dependent on the presence of antigen. IgA lymphoblasts migrate to fetal isografts of small intestine transplanted underneath the kidney capsule of an adult mouse.¹⁸ When rats primed by intracolonic injection of cholera toxin were challenged 14 days later by injecting antigen into a surgically isolated loop of jejunum with an intact blood and lymph supply, blast cells appeared in comparable numbers in the stimulated intestinal loop and in the unstimulated jejunum. By cannulating and draining the thoracic duct, the researchers confirmed that antigen stimulation of memory cells in the isolated jejunal loop resulted in the production of lymphoblasts that entered the lymph and circulated to populate the entire jejunum.¹⁷ Although memory cells recirculate to populate the mucosal tissues, general mucosal dissemination of lymphoblasts committed to IgA synthesis does not occur after secondary challenge. When thoracic duct lymphoblasts from animals both primed and challenged by intracolonic injection of cholera toxin were transferred into naive recipients, large numbers of antibody specific plasma cells populated the colon but there were virtually

none found in the jejunum. These studies suggest that the lymphoblasts can distinguish between high endothelial cell receptors in the colon and jejunum indicating that the colon receptors have their own 'homing' specificity. The presence of antigen, however, profoundly affects the magnitude and persistence of the response due to antigen-driven clonal expansion of antibody forming cells and their precursors.¹⁶

Intestinal fluid immunoglobulins

A specialised form of IgA, secretory IgA, represents the predominant antibody isotype in intestinal fluid where the relative concentrations of the major immunoglobulin isotypes mirror their relative distribution in the antibody-containing cells of the intestinal mucosa.³ Unlike IgA in the serum and cerebrospinal fluid which largely comprises 7S monomers, secretory IgA is an 11S dimer made up of two IgA monomers joined by a covalently linked peptide named J chain.¹⁹ Both IgA and IgM plasma cells synthesise J chain which greatly enhances the binding of an additional polypeptide, secretory component (SC), thereby completing the assembly of secretory IgA and IgM molecules. The details of secretory IgA structure have recently been reviewed.¹⁵ J chain is an acidic peptide of 137 residues which may be involved in the intracellular polymerisation of IgA. One molecule of J chain joins two IgA monomers whereas three or more J chain molecules are involved in forming the IgM pentamer within the plasma cell before secretion.

Secretory component (SC) is a glycopeptide molecule synthesised by epithelial cells in secretory glandular tissue and mucosal surfaces. In some mammalian species, but not man, hepatocytes also synthesise and display SC. Secretory component exists in three molecular forms; as a membrane protein expressed on the outer surface of epithelial cells, it acts as a receptor for polymeric immunoglobulins. Secretory component forms part of secretory IgA and IgM molecules in mucosal fluids where it is also found as a free glycoprotein. Secretory component interacts with a specific binding site on the Fc region of SIgA and IgM, stabilises their quaternary structures, and increases their resistance to proteolytic digestion.³ Structural studies of human SC reveal a characteristic composition, comprising 549 to 558 amino acids and a high carbohydrate content (20%). Twenty cysteine residues create 10 intrachain disulphide bridges in a single polypeptide chain that has five regions of remarkable homology. The fifth homology region contains an additional labile disulphide bond that is involved in the formation of a disulphide bridge linking SC to the two

chains of one of the monomers in dimeric IgA. The homology regions of SC are structurally related to the variable domain of immunoglobulin light chains and probably have a similar tertiary structure. Since the secondary structure of J chain also shows immunoglobulin like folding, the interactions of α , μ and J chains with SC may be based on the complementaries of their domain-like structures.²⁰ Both the additional peptides SC and J chain bind covalently to the Fc region of IgA and IgM.

The constant domains of the α chains of the two subclasses of IgA, IgA₁ and IgA₂ are very similar. The C terminus of the α chain resembles that of the μ chain and extends further than the C termini of the γ , ϵ and σ chains of IgG, IgE and IgD strongly suggesting that this extension is implicated in the ability of IgA and IgM to form polymers.¹⁵

The predominant structural differences between IgA₁ and IgA₂ occur in the hinge region where IgA₁ has a unique sequence of 13 amino acids that are susceptible to cleavage by highly specific bacterial IgA proteases. The corresponding sequence is lacking in IgA₂ molecules which are therefore resistant to nearly all known bacterial proteases with the exception of a clostridial IgA protease that cleaves a peptide bond in the hinge region of both IgA₁ and IgA₂ of the A2m(1) allotype.²¹

Plasma cells producing 10S IgA are found in all mucosal tissues especially in the intestinal lamina propria whereas plasma cells in the bone marrow, spleen and peripheral lymph nodes secrete monomeric (7S) IgA. There is also a selective distribution of cells producing the IgA₁ and IgA₂ subclasses. IgA₁ plasma cells form the majority in all tissues apart from the colon but there is marked variation in the ratio of IgA₁ to IgA₂ plasma cells between lymphoid tissues from different sites. That IgA₂ is the preponderant subclass in the colon may relate to local stimulation by endotoxins from commensal gram negative organisms which mainly induce an IgA₂ subclass antibody response resistant to bacterial proteases.¹⁵

Secretory IgA function

The protective role of secretory IgA against a variety of foreign antigens, including food antigens bacteria viruses and toxins, is well established. S-IgA blocks the access of potentially allergenic molecules derived from food or drugs. S-IgA mediated responses at mucosal surfaces to locally encountered antigens are readily detected but prolonged topical exposure to protein or particulate antigens is required to induce serum antibodies.²² Antibodies of all three major isotypes specific for food and microbial antigens are

detectable in the sera of normal individuals. They are mostly IgA₁ class and very high serum titres are found in IgA deficient patients. S-IgA may also block reaginic sensitivity reactions at the surface of the intestinal epithelium as reported for nasal epithelium. Because some dietary antigen is clearly absorbed by normal subjects, the importance of S-IgA antibody may lie in reducing the amount of antigen that gains access to the lamina propria.

S-IgA can neutralise biologically active antigens, including viruses and toxins and enzymes, prevent adherence of bacterial pathogens to epithelial surfaces and enhance the antibacterial efficiency of other immune effector systems. The effectiveness of S-IgA as a neutralising antibody against viruses is shown by the responses to the Sabin oral live poliovirus vaccine where protection correlates with levels of secretory antibody.²³ The four antigen combining sites of S-IgA also allow it to function efficiently as an agglutinin and the capacity of S-IgA to protect by inhibiting bacterial adherence to the mucosal epithelium is exemplified by S-IgA antibodies to *Vibrio cholera*. There is also evidence that IgA has bacteriocidal potential in cooperation with complement and lysozyme and S-IgA can opsonise bacteria as outlined below.

Unlike IgM or IgG, however, secretory IgA does not recruit the powerful mediator systems that are activated in systemic immune responses. In particular, sIgA-antigen complexes do not activate the classical or alternative pathways of complement.²⁴ Chemically aggregated IgA in high concentration does activate the alternative pathway but the biological relevance of the finding is questionable.

IgA and IgA-containing immune complexes are capable of interacting with neutrophils, mononuclear phagocytes, epithelial cells lining mucous membranes, T, B and NK cells. The Fc receptors for Cα2 domain of polymeric and monomeric IgA and for sIgA of both subclasses that are displayed by subpopulations of neutrophils and mononuclear phagocytes increase in number when these cells are exposed to high levels of IgA. These findings may explain the enhanced phagocytic activity of mucosal neutrophils compared to those from the circulation.²⁵ The functional significance of Fcα receptors on phagocytes is uncertain. Earlier reports suggested that IgA had little capacity as an opsonin, but recent studies indicate that particles coated with IgA are readily phagocytosed by neutrophils from the oral cavity.²⁵ Neutrophils may also clear IgA complexes from the blood. Macrophages in colostrum and milk contain large amounts of S-IgA suggesting that they provide the means for transporting S-IgA into the lower gastrointestinal tract of breastfed infants. There is also evidence that IgA can 'arm' neutrophils

and Th cells to provide cell mediated antibacterial activity.²⁶

The secretory component (SC) displayed on the basolateral membrane of mucosal epithelial cells acts as a receptor for polymeric IgA and IgM by binding to a J chain-related site. The SC-polymeric IgA complex is internalised by the epithelial cell and transported as a vesicle to its apical surface where the SC-IgA complex is released.²⁷ Although human hepatocytes do not express surface SC they do have surface membrane receptors specific for asialoglycoproteins called hepatic binding proteins that bind human polymeric IgA₁ and to a lesser extent monomeric IgA₁. IgA₂ does not inhibit this interaction. The human liver rapidly clears S-IgA₁ from the circulation indicating that the hepatic binding protein acts as a receptor for S-IgA₁ uptake.²⁸ Unlike those species that have hepatocytes bearing SC, humans do not transport large quantities of circulating IgA across the hepatocyte and into the bile. In human bile, only about 50% of the polymeric IgA is derived from the plasma.

Regulation of S-IgA immune responses

Many characteristics of the S-IgA system clearly distinguish it from systemic immunity. GALT contains all the cells required for local responses and is constantly exposed to antigenic stimulation and to biological active bacterial components such as endotoxin, but a number of negative regulatory factors suppress the induction of a localised immune response. There is selective priming of GALT B cells leading to marked restriction of the isotype potential of B cells to IgA expression.²⁹ Endogenous gut endotoxin appears to enhance the host's resistance to gram negative infections possibly by interacting with lymphoreticular cells in GALT resulting in induction of innate host immunity to infection.

The IgA response to antigens including carbohydrate antigens is controlled by T cells. Peyer's patch T cells stimulated by Concanavalin A help IgA expression but suppress IgM and IgG in LPS-driven B cell cultures whereas similarly treated splenic T cells suppress all three isotypes.³⁰ Oral administration of soluble or particulate antigens induces Th cells in GALT which are specific for the IgA isotype and probably account for the IgA isotype response that results from oral immunisation.

Two separate modes of T cell regulation for the IgA response apparently exist in GALT. T cells may induce B cells to switch isotypes (Tsw)³¹ by promoting the switch of B cells bearing surface IgM (sIgM') to cells expressing surface IgA (sIgA) – events that are influenced by factors released by activated T cells including interleukins 4 and 5. A second mode of T

cell regulation of IgA responses may involve Th cells bearing Fc receptors for IgA (Fc α) which preferentially interact with sIgA⁺B cells and differentiate them into IgA-producing plasma cells.³² In mice, Fc α receptors (Fc α R) are also found on some Ts cells that can release the Fc α R as an IgA-binding factor (IBF α) resulting in suppression of IgA synthesis in pokeweed mitogen driven spleen cell cultures. On exposure to IgA, activated Fc α R⁺T cells released IBF α and suppressed IgA responses. Supernatants of Fc α R⁺ hybrids created by fusing Peyer's patch Th cells with a T cell lymphoma line, supported antigen-dependent IgA responses in cultures of normal Peyer's patch B cells whereas Fc α R⁻ hybrid supernatants did not. When IBF α was purified from the supernatants, high concentrations suppressed T cell-dependent IgA responses while low levels of IBF α enhanced IgA responses.³³ These results indicate that T cell selection and induction of sIgA⁺ B cells involves Fc α R and that high concentrations of IBF α have a paradoxical suppressor effect. Whether these effects derive from different concentrations of the same IBF α molecule or whether, as has been shown for IgE, there are two classes of IBF, one suppressor the other helper, that differ in their carbohydrate moieties,³⁴ has not been established. Thus clones of T cells may produce both surface Fc α receptors and a secretory form(s) of IBF α which markedly influence the responses of sIgA⁺ cells in a complex network to generate IgA specific immunoregulation at exposed mucosal sites involving selective homing mechanisms for redistribution of IgA-committed cells.

T cell networks and oral tolerance

Two separate immune responses follow oral administration of antigen in large doses; S-IgA antibodies are generated at mucosal surfaces and unresponsiveness to the same antigen – that is, tolerance, develops systemically. The simultaneous generation of Th and Ts cells specific for the same antigen implies another level of control which is apparently provided by the contra-suppressor T cell (Tcs). Indeed Tcs, which are enriched along with Th cells in Peyer's patches, can abolish oral tolerance when adoptively transferred to an animal orally immunised by the same antigen. IgA isotype-specific Tcs cells have recently been reported in Peyer's patches. These Tcs bind to immobilised Fc α R⁺ helper cells and, when adoptively transferred, also abolish oral tolerance.³⁵

Although circulating immune complexes³⁶ and anti-idiotypic antibodies³⁷ have also been proposed as mechanisms mediating oral tolerance, the evidence suggests that a T cell suppression network plays the major part in the downregulation of systemic immune responses.

Mucosal cellular responses

Studies of effector cell populations in isolated lamina propria mononuclear cells have identified marked differences in their relative frequency, functional state and phenotype in comparison to corresponding cell populations in the peripheral blood. Lamina propria lymphocytes show enhanced proliferation after exposure to interleukin 2 (IL-2), a much higher proportion of IL-2 receptor-bearing cells in the Th and Ts subpopulations and an increased proportion of T cells expressing class II antigens of the major histocompatibility complex. Moreover, IL-2 receptor mRNA is detectable in unstimulated lamina propria lymphocytes. But there is no evidence of IL-2 mRNA expression. Peripheral blood lymphocytes do not express either mRNA.³⁸ Mucosal T cells therefore selectively express some of the genes associated with T cell activation resulting in 'partial' activation or 'primed' mucosal T cells that may result from unique, possibly environmental activation signals in the intestine or altered regulation of T cell function at mucosal surfaces.

Mucosal cell mediated cytotoxicity unrestricted by the MHC is also markedly distinct from that found in peripheral blood. Natural killer cells represent 2–3% of dispersed lamina propria cells, as identified by the NKH-1 monoclonal antibody, but are Leu 11⁻.³⁹ Natural killer activity is very low or undetectable in unseparated lamina propria cells^{40,41} but can be discerned in purified NKH-1⁺ cells.³⁹ Mucosal lymphokine activated killer (LAK) cells also express a different phenotype to those in peripheral blood being T11⁺, NKH-1⁻, Leu 11⁻, T4⁻, T3⁻, T11⁻, and T8⁻.^{39,40} and high levels of cytotoxicity to cell lines and to freshly isolated cells can be generated after exposure of lamina propria lymphocytes to IL-2 in culture for 72h.^{40,41} Non-specific cytolytic cells activated by lectins which are also present may well be a variant of the LAK cell. Although the numbers and cytotoxic activity of mucosal NK cells are very limited, it remains possible that other NK cell capabilities, including resisting viral infection, and immunoregulation may be relevant to mucosal homeostasis. Lymphokine activated killer cells lyse solid tumour cells *in vitro* but whether the conditions exist to generate this population *in vivo* is unclear. Markers of T cell activation are markedly increased in lamina propria lymphocytes compared to those from peripheral blood. These include proliferative responses to IL-2, IL-2 receptor expression and function and expression of class II MHC antigens but there is no enhancement of IL-2 synthesis. Mucosal NK cells and other Fc-bearing cells including macrophages may mediate antibody-dependent cytotoxicity (ADCC). Antibody-dependent cytotoxicity has been

shown in lamina propria cells but its importance to mucosal inflammation remains to be assessed.

An indirect measure of *in vitro* primed cytotoxic T cell activity that is not MHC restricted may be obtained using antibodies to the CD3 component of the human T cell receptor.⁴² High levels of cytotoxicity were found in lamina propria cells from histologically normal colon possibly reflecting the exposure of the mucosa to consistent and diverse antigenic challenges. These effector cells display the CD2⁺, CD3⁺, CD8⁺, CD4⁻, CD16⁻ and Leu 7⁻ phenotype and the pattern of target cell specificity may be determined by the CD45 antigen which is coupled to the trigger process for NK lysis of some target cells.⁴³ This cell population may represent *in vitro* primed cytotoxic T cells reactive against undetermined antigens including viruses.

Conclusion

Clinical research involving the detailed study of individual patients has made many important and original contributions to understanding of the physiology of the intestinal immune system in health and disease. In this context, the study of individual patients suffering from alpha chain disease (α CD) represents a paradigm. The importance of diffuse, small intestinal lymphomas called Mediterranean lymphomas (ML) as a cause of malabsorption in the Middle East had been recognised for many years, but understanding was limited to descriptive accounts of the morphology of the cellular infiltrate as seen in stained paraffin sections of intestinal tissue. When individual patients in Paris⁴⁴ and at Hammersmith⁴⁵ were investigated, it was discovered that the diffuse cellular infiltrate represented a monoclonal expansion of benign appearing plasma cells which synthesised, and usually secreted, a marker protein comprising a heavy chain fragment of IgA, devoid of light chains. Analysis of the Fc α fragment found in serum, urine and intestinal fluid revealed that it bound secretory component (SC), providing the first evidence for the binding site of SC to the IgA dimer. Left untreated, α CD progresses to a frankly invasive intestinal lymphoma. The discovery of α CD, has provided a unique and important opportunity to study the natural history of a B cell lymphoma as well as offering the prospect of early diagnosis by detection of the marker protein and of cure.⁴⁶

The molecular analysis of immune and inflammatory events at the mucosal surface in human beings has been greatly enhanced by the improved access to tissue provided by developments in fibre-optics and in ultrasound, microassay systems including HPLC, fluorimetry, monoclonal antibodies and DNA probes, and the use of recombinant DNA

technology to engineer micro-organisms not only to produce large quantities of previously unavailable natural peptides in pure form but also to create recombinant vaccines for oral immunisation against intestinal infection and infestation. Besredka's vision of oral vaccines is now being realised.

*Division of Clinical Sciences,
John Curtin School of Clinical Research,
Australian National University,
Canberra.*

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