D. J. du Plessis Lecture

Mucus: Slippery, sticky, but sweet and satisfying

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It is indeed an honour to present the 29th D. J. du Plessis lecture in memory of the man who was Chair and Head of the Department of Surgery at the University of Witwatersrand (Wits), a teacher, an academic, and in the latter part of his career between 1978 and 1983, Vice-Chancellor of Wits (an unenviable position during turbulent times in the history of South Africa).[1]

In a 2002 dedication to D. J. du Plessis, Professor J. A. Myburgh, a former Head of the Department of Surgery at Wits, compared Jan Smuts's wartime habit of carrying a Greek New Testament during the South African war to maintain his Greek to that of Du Plessis, who studied basic surgical sciences in the desert whenever he could, enabling him to proceed to postgraduate training in surgery immediately after demobilisation in 1946.[2] This kind of ambition and passion for the acquisition of knowledge is both inspiring and astonishing. I take great pride in having grown academically in a similar environment here at the University of Cape Town (UCT), where many of my own mentors and colleagues embody the same spirit and passion for knowledge and service to humanity.

The public perception of mucus as a biological secretion is usually amusement coupled with distaste, the prototypical image being that of a footballer expectorating on the pitch, or the outpouring of viscous nasal secretions associated with the common cold! Even classic literature reflects the impact of mucus in our lives; famous writers and playwrights have used mucus as a metaphor in literature, for example the reference to 'the snotgreen sea' in James Joyce's masterpiece Ulysses, and 'the significance of snot and other effluvia' in the plays of William Shakespeare.[3]

Mucus research as a career

The details of how and why I came to be a mucus researcher is a story that will be told on another occasion. Suffice to say that it was an unplanned visit to the University of Cape Town Medical School during a holiday in the Cape in 1979, and a chance meeting with Professor Wieland Gevers (a rare combination of a gifted mind, and a caring human being), then head of Medical Biochemistry, that changed the course of my life. My postgraduate career began under the watchful eyes of Professor Gevers and the magnanimous Emeritus Professor Rosemary Hickman, in the research laboratory of the Department of Surgery, chaired by Professor John Terblanche. This chance meeting with Professor Gevers and the course of my life subsequent to that meeting bring to mind the idea in Milan Kundera's The Unbearable Lightness of Being,[4] that every step one takes in life has an infinite number of possibilities. It was during that period (in 1984) that Professor Gevers was awarded the Distinguished Teacher Award, and it was a humbling experience for me to be the 'other' distinguished teacher in Medical Biochemistry when UCT bestowed the award upon me in 2000.

It is to the eternal credit of my hosts that even during those dark days of apartheid, my racial background and other mundane issues such as the requirement of a black South African to obtain a governmental permit to attend an institution reserved for whites did not deter them from their goal of having me as a postgraduate student in the Faculty of Medicine at UCT. Rosemary Hickman, one of the most selfless human beings I have met, was kind, welcoming and patient, knowing all too well the difficulties of working with mucus, a very new research field in South Africa.

Mucus and mucins

Mucus

Mucus, a visco-elastic secretion with both semi-solid and flow properties,[5] forms a continuous, insoluble adherent gel layer in the gastrointestinal tract, which protects the underlying mucosa from the hostile environment of the lumen.[6,7] Mucus is a complex secretion, mainly consisting of water but also containing proteins, lipids, nucleic acids, cell debris, food particles, ions and mucins, the mucous glycoproteins to which the stickiness of the secretion is attributed.[8]

Besides acting as a lubricant on epithelial surfaces, versatility and specialisation are features of mucus. For example, the mucus gel barrier on the mucosal surface of the stomach protects it from the shear forces associated with digestion and the potency of hydrochloric acid;[9] the muco-ciliary blanket of the respiratory tract traps and returns to their origin inhaled dust particles and other foreign impurities, to protect the alveoli; colonic epithelium must be shielded from hard faecal materials and bacteria; the plug of cervical mucus at

the mouth of the cervix protects against the entry of bacteria and facilitates the movement of sperm during the mid-cycle; saliva aids in the lubrication and homogenisation of chewed food; gallbladder mucus protects the underlying epithelium against a concentrated mixture of surface-active chemicals. In each instance, the general but variable properties of mucus are exploited differently to fulfil the special function that is required.[10] In certain diseases such as pseudomyxoma peritonei, the mucus in the abdominal cavity is semi-solid and cannot be solubilised.[11]

Mucins (mucous glycoproteins)

There are two types of mucins, the secreted[5] and the trans-membrane types.[12,13] The secreted mucins impart gel-forming properties to crude mucus secretions of the epithelial surfaces of the internal tracts of the body.[5] The four major secreted gel-forming mucins are MUC5AC (stomach[14] and airways[15]), MUC5B (airways[16] and gallbladder[17]), MUC2 (colon[18]) and MUC6 (stomach[19]), the genes of which are clustered on chromosome 11. There are a whole host of trans-membrane mucins, from which I have chosen MUC1 and MUC4 for this presentation.

The two postulated structural models for gel-forming mucin

Mucins are elongated, rod-like molecules called subunits (monomers) comprising a protein backbone to parts of which oligosaccharide side-chains are attached (the bottle-brush regions), the rest being 'naked' or free of carbohydrate. The carbohydrate portion comprises more than 70% of the molecule. The prequisite for there to be an effective gel on the epithelial surfaces of the internal tracts of the body is that the mucin components of the crude gel have to be in a polymeric form. To achieve this, the mucin subunits must join at their 'naked' regions by disulphide bonds to form large polymers. The first ever model for mucin structure and conformation was postulated by Adrian Allen and his group in Newcastle upon Tyne, UK, who proposed the windmill formation, in which four subunits are joined to one another by disulphide bonds through an inter-linking 'link' protein[5,20] (Fig. 1). In 1983, Ingemar Carlstedt of Lund, Norway, and John Sheehan of Manchester, UK, proposed that mucin subunits are joined end-to-end by disulphide bonds in random coil fashion[21] (Fig. 2). This controversy was never successfully resolved, and part of my PhD in the laboratory of Adrian Allen was an attempt to resolve this structural controversy in mucin biochemistry.[22] For mucin biochemists it is sufficient that subunits join to form polymers, and when isolated in an un-degraded form from crude mucus secretions in appropriate solvents, their conformation is most likely to be that of a random coil.[21]

A lesson for a trainee scientist

This experience, in which I was expected to undergo a paradigm shift with respect to a model of mucin structure in the short space of a few years as a postgraduate student, is for me at the heart of a controversy in which there has been a longstanding criticism of the scientific method and science in general. It was an important lesson for a developing scientist, and is proof against the unfairness of the perception in certain quarters that scientists stubbornly and rigidly cling to pet ideas, a claim made by creationists (and
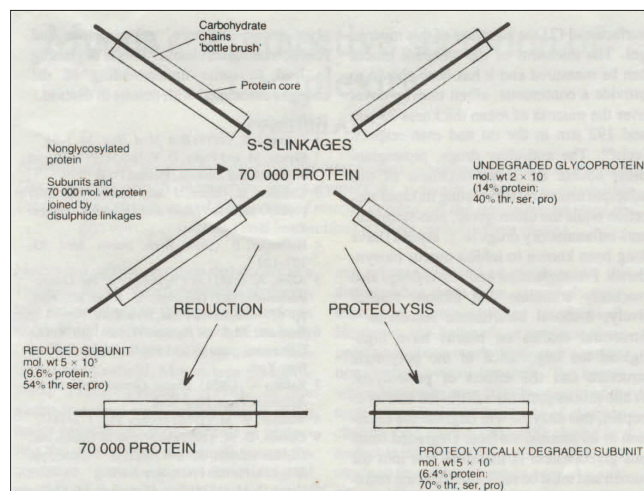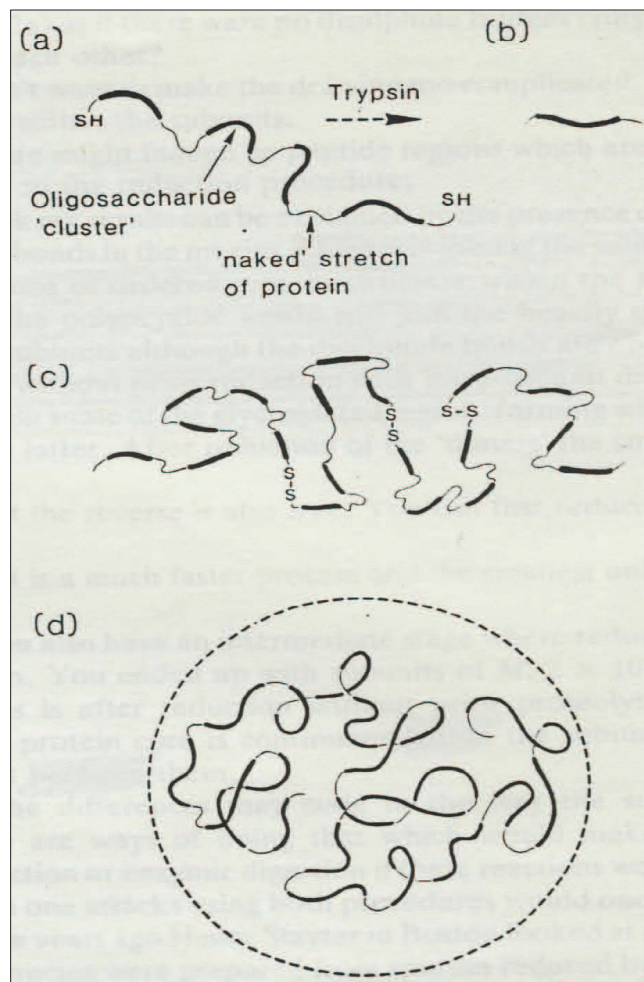

Fig.1. 'Windmill' model of mucin structure.[5]

Fig. 2. Random coil model of mucin structure.[21]

the proponents of intelligent design) in the current debates raging between scientists and evolutionists. Creationists have perpetually accused scientists of claiming to have access to the absolute truth. However, my own experience regarding the biochemical structure of mucin, together with the views of other world-renowned scientists, challenges this perception. According to Robert Ehrlich, Professor of Physics at George Mason University in Virginia, USA, science is use-

ful because it cannot explain everything – 'It is the tentative nature of science and the ability of future evidence to prove current theories wrong that constitutes its great strength'.[23] It was Mary Leakey, that remarkable palaeontologist in East Africa, who said 'Theories come and go, but fundamental data always remain the same'.[24] Lawrence Slobodkin of the State University of New York has been attributed by Edward O Wilson of Harvard with the statement that 'nature defeats theory'.[25] There are no absolute truths in science, and theories, even dearly held ones, are vulnerable to new ideas and the increased sophistication in technology.

So, 'can science and God ever get along?', to paraphrase the title of one of the many recent articles on this subject (by Tim Hames in *The Times* of London, 26 May 2008[26]). To their credit, some creationists, such as Rabbi Harold Kushner, refuse to polarise this debate, stating that the universe was built on and governed by the 'laws of nature'. The fact that we can to a large extent live orderly lives, is because these laws are precise and reliable and do not change. Our human bodies are miracles, not because they defy these laws of nature *but precisely because of them*.[27]

Mucus and mucins in disease

The gastrointestinal tract

There is a dynamic balance on the mucosal surface of, for example, the stomach. The degradation of mucin polymer, in the surface gel by pepsin, resulting in mucin subunits being released into the gastric lumen, is accompanied by a secretion of mucin from mucosal epithelial cells, thus ensuring that the mucus gel on the surface retains its thickness and protective properties.[28]

A greater subunit-to-polymer ratio in the gel on the gastric mucosal surface in peptic ulcer disease suggested that a weak gel was associated with ulceration of the stomach.[29] Our laboratory also showed, in a well-established pig gastric ulcer model, that the reproducible ulcer that resulted in the pars oesophagea after bile duct ligation was associated with degradation of the gel on the gastric mucosal surface.[30] Although *Helicobacter pylori* was detected in the tissue, its presence was not associated with ulcer formation.[31] Similarly, patients with carcinoma of the stomach had only approximately 6% of polymeric mucin in gel scrapings from their mucosa, again associating a defective gel layer with disease. Besides the mucins being mostly degraded in carcinoma of the stomach, we reported the appearance of a Mr ~ 55 - 65 kDa fragment that fractionated with mucin during its isolation and purification of the crude gel.[32] This factor has now been identified as α-1 acid glycoprotein (orosomucoid), found in parietal cells of the stomach and in intestinal metaplasia (N Chirwa *et al.*, unpublished data).

MUC2 is the gel-forming and predominant mucin in the human colon, and the development of the majority of colorectal cancer (CRC) is associated with a diminished expression of MUC2 in the tumour cells,[33] and MUC2 knockout mice have been shown to develop cancers.[34] On the other hand, Bresalier *et al.*[35] showed that mucinous colonic carcinomas with cancer cells that make abundant mucin are more likely to metastasise and that inhibition of mucin synthesis is associated with a reduction of metastatic potential. A defective polymerisation of secreted mucin has been reported in ulcerative colitis and anti-MUC1 antibodies have been detected in the sera of patients with ulcerative colitis, sug-

gesting that in such an inflammatory condition, antibodies to colonic epithelial cells could contribute to their injury.[36,37] The presence of the intestinal mucin MUC2 in intraductal papillary mucinous tumours (IPMT) gives a worse prognosis than its absence.[38] MUC2 was also found in the sputa of patients with tuberculosis in a study done in our laboratory.[39]

HIV-AIDS

Work in our laboratory has also shown that crude saliva and purified salivary MUC5B and MUC7 inhibit the HIV-1 virus[40] and the pox virus[41] in an *in vitro* assay. Crude breast-milk[42] and cervical mucus[43] do not inhibit the virus, but the purified mucins from these secretions do. Salivary mucin from patients (with different CD4+ counts) infected with HIV also does not inhibit the virus in an *in vitro* assay. These mucins from infected patients have variable charges, probably due to altered glycosylation patterns which could affect their viral binding properties, unlike the normal mucins (unpublished).

The carbohydrate oligosaccharide chains of mucins and their association with disease

Most of the alterations in mucins in disease occur in the carbohydrate side-chains or oligosaccharides, which can have anything from 2 to 22 monosaccharides and are attached to the protein core through an O-glycosidic linkage.[5] These oligosaccharide chains harbour a whole host of antigens, including blood group antigens. Certain diseases are associated with alterations in the sequence of monosaccharides, or loss of certain sugars (hypoglycosylation), or the presence of 1 - 3 sugars as in the case of truncated antigens, for example, T antigen (see below). A definitive study in 1982 by Boland *et al.*[44] showed that normal colonic mucin, as detectable by a lectin, Dolichos biflorus agglutinin (DBA), was different from that in cancer, which was detectable by peanut agglutinin lectin (PNA), suggesting that in disease there was an alteration in the pattern and sequence of glycosylation of mucins.[45] Since then, much has been reported in the literature on altered glycosylation of mucins in CRC which presents as new and different epitopes. It has been shown that these alterations are found in both secreted and trans-membrane mucins.

The truncated antigens

It is known that oncogenic changes are associated with changes in glycosylation in glycoproteins and glycolipids,[45] generating new antigens exploitable as laboratory diagnostic markers, among which are T, Tn and sialyl-Tn antigens, in a variety of cancers.[46] They are referred to as pan-carcinoma antigens, having a remarkable cancer-specific expression pattern.[47] The sialyl-Tn epitope is one of the most specific tumour antigens described so far, being highly expressed on many adenocarcinomas but having a very limited expression in normal adult tissues.[48,49] Expression of sialyl-Tn is an independent predictor of poor prognosis in colon cancer,[50] with a significant correlation between expression and 5-year survival and even disease-free survival.[51] The presence of T antigen within these structures in the majority of colorectal cancers suggests that this antigen favours liver metastasis and that its expression in primary CRC is a significant risk factor for the development of liver metastasis.[52] The expression of Tn is associated with a shortened 5-year disease-free interval, positive lymph node status and an increased histological grade.[53]

Higher expression of T antigen than sialyl-Tn has also been reported for HNPCC (hereditary non-polyposis colorectal cancer).[50] The increased expression of T, Tn and sialosyl-Tn (all onco-developmental cancer-associated antigens in the colon) suggests that incomplete glycosylation is characteristic of mucins in colon cancer.[51]

The trans-membrane mucins

MUC1 and MUC4

The trans-membrane mucins are built on the same principle as the secreted mucin subunit, with the appearance of a bottlebrush structure. MUC1, the first trans-membrane mucin and the first mucin to be cloned from mammary carcinoma tissue but also found in adenocarcinomas of ovary, lung, prostate, colon and pancreas, occurs on the apical surface of most epithelial cells[54] (Fig. 3). During tumorigenesis of the breast, MUC1 is hypo-glycosylated (even on breast cancer stem cells[55]), and detectable by SM-3 antibody, different to that in normal tissue which reacts with HMFG-1 antibody. It is over-expressed in breast cancer, the cell loses its polarity and the large size of the MUC1, together with its high charge density, aids in metastasis. The exposure of new antigens renders them recognised as non-self and elicits a cytotoxic T-lymphocyte response.[55] The transformed cell escapes immune surveillance by shedding the excess MUC1
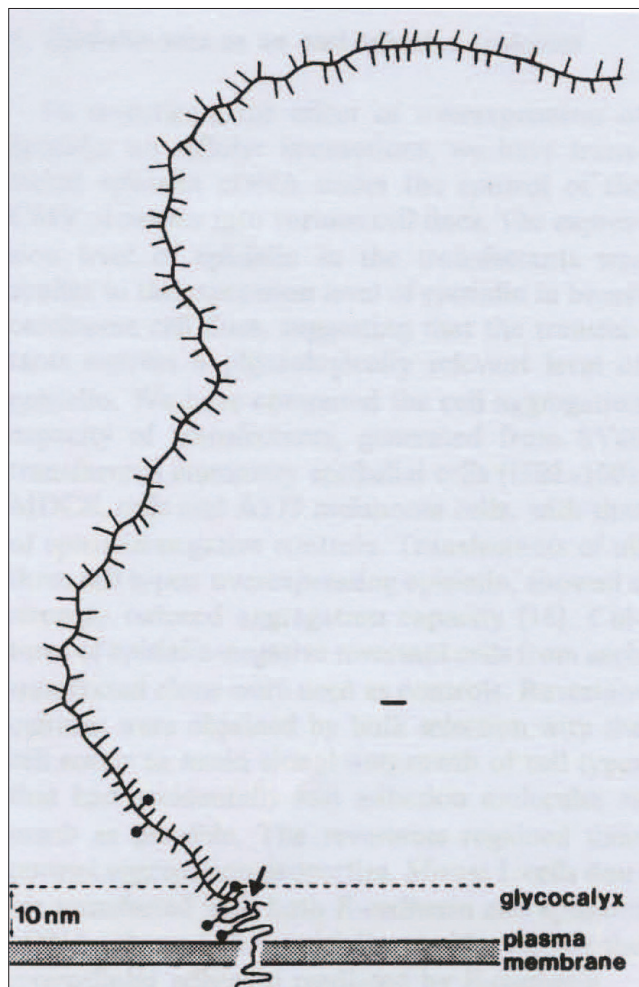

Fig. 3. MUC1 – a trans-membrane mucin.[54]

from the cell surface, which diverts the immune attack from the T lymphocytes.

Compared with other tumour markers, MUC1 is the best tumour antigen as a diagnostic aid for breast cancer.[56] If it is elevated in a patient with apparent localised breast cancer but remains high postoperatively, occult metastasis is likely.[57] Altered and variant glycosylation together with over-expression make it a target for immunodiagnostic and therapeutic measures,[47] within the concept of active-specific immunotherapy (ASI) and vaccine development.

The discovery of MUC1 caused a flurry of excitement in the mucus world with dozens of articles and reviews on the subject, with it even being referred to as 'the renaissance molecule'[58] or a multifaceted glycoprotein.[59] MUC1 is over-expressed in cancer of the prostate gland, to which L-BLP25, a synthetic liposomal vaccine, has been designed to target a specific region of MUC1.[60] MUC1 gene polymorphisms are associated with susceptibility to chronic atrophic gastritis and intestinal metaplasia[61] and an increased risk of gastric carcinoma,[62] and a single-nucleotide polymorphism (SNP) in MUC1 has been reported in ulcerative colitis.[63] Bile salts activate the expression of MUC1 in oesophageal carcinoma.[64]

MUC4, on the other hand, is not expressed in the normal pancreas or in chronic pancreatitis, but the activation of its expression is observed in the early steps of pancreatic carcinogenesis.[65] More recently MUC4 has shown great promise as a tumour marker since its expression in tumours of varying stages and types have shown it to have potential for clinical use in the diagnosis and/or management of pancreatic, lung, breast, gallbladder, salivary gland, prostate and ovarian cancers. MUC4 has shown to be a good candidate marker for early diagnosis of pancreatic cancer in fine-needle aspirates, exhibiting 91% sensitivity and 100% specificity.[66]

Active specific immunotherapy (ASI) and vaccine development arising out of MUC1 research

The concept of ASI is to immunise with a defined antigen, presented in an appropriate manner, and thus actively induce an immune response specifically to that antigen. ASI utilises cancer vaccines to stimulate the patient's own immune system to attack the aberrantly glycosylated MUC1.[67]

Giants

D. J. du Plessis has been called a surgical giant in southern Africa,[68] and my career at the University of Cape Town has been built on the shoulders of people of similar stature (Professors Gevers, Hickman and Terblanche). The tradition of good leadership in the Department of Surgery continued after the retirement of John Terblanche, who was succeeded by Professor David Dent, who I thank for his leadership, guidance and mentorship. The current Chair of the Department of Surgery, Professor Delawir Kahn, has made research a priority in the Department of Surgery and his passion in this regard never fails to inspire staff to greater heights.

Conclusion

I have been enriched by a life in science. One of the best definitions of the activity of science was by Niels Bohr, who won

the Nobel Prize in 1922 for his services in the investigation of the structure of atoms and from the radiation emanating from them.[69] Bohr said that science was a crude, pragmatic, effective way of understanding the world, much like washing up dirty crockery after dinner, resulting in clean dishes but a dirty drying cloth and dirty water. Philosophy, in compari-son, was a very similar activity, but without the water![70]

My family tires of my continually extolling the virtues of science and scientific activity. I must confess my relief that my daughters Sumaya and Raissa have chosen scientific disciplines as careers. While I agree with that 20th-century icon of physics Richard Feynman, who won the Nobel prize in 1965,[71] that children shouldn't be pushed in any direction and should be allowed even truck driving as a career if they so wish and are happy doing it, like Feynman (who expressed his shock that his son Carl, at the Massachussets Institute of Technology, chose philosophy as a career),[72] I too would have had reservations had my children chosen non-scientific careers.

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