INTRODUCTION

This EU grant has helped support three meetings, the 6th Meeting on Mucins in Health and Disease (previously known as the International Workshops on Carcinoma-Associated Mucins) held in Cambridge, UK in 2000 and two smaller workshops on Carbohydrates and the Immune Response held in Greece. The series of Workshops on Carcinoma-Associated Mucins are the only international meetings devoted solely to mucin glycoproteins and as such they attract participants from all over Europe as well as the rest of the world, including Australia, Japan and the USA. The EU grant has allowed these meetings to remain in Europe, making Europe the focus of mucin research. The smaller two meetings in Greece covered the rapidly expanding field of the involvement of carbohydrates in the immune response and grew out of the larger mucin meetings. All three meetings brought together scientists involved in many disciplines, with a range of expertise covering the biosciences from synthetic chemistry to cell biology but all with a converging interest in mucins.

Scientific

Mucins are proteins, which carry a large amount of sugar attached through oxygen (O-linked) to the protein core. The epithelial mucins are expressed by the cells that line the tubes and glands in some tissues of the body, for example the stomach, colon and ducts of the breast. The carcinomas arising from such tissues also express these mucins but at a higher level and there is a marked change in the composition and density of the sugar-side chains, making these molecules potential targets for Active Specific Immunotherapy. The expression and content of the sugar side-chain repeats of epithelial mucins is also altered in disease such as asthma and cystic fibrosis, and mucins can act as sites of attachment for some bacteria. Fifteen epithelial mucins have now been identified and at least five of these are tethered to the cell membrane, while the others are secreted by the cell. Each mucin has a different profile of tissue expression but a single tissue, for example the colon, can express a number of different mucins. One characteristic of all the epithelial mucins is that they contain tandemly repeated amino acids and this domain contains multiple sites for the attachment of the sugars.

Other types of mucins are expressed by blood cells and are involved in the immune response either by binding molecules found on pathogens, or by being involved in the movement of white blood cells to areas of inflammation.

The first and third events in this programme were on the influence of carbohydrates on the immune response, a topic which was initially, covered by the larger mucin meetings. However, it has become apparent that this rapidly developing field requires a series of more specialised Workshops. The immune system was developed to fight invading organisms, viruses and bacteria, and consist of two arms – The innate immune system and the adaptive immune system. The adaptive immune response is only found in animals with backbones and is responsible for the...
production of antibodies and special cells (T cells) that have the ability to kill virus infected tissue or help in their destruction. Although it has been known for a long time that antibodies can recognise sugars, it is only just starting to become clear that T cells can also recognise sugars attached to proteins or fats when presented to the T cell by specialised molecules on antigen presenting cells. The innate immune system is more ancient in evolutionary terms and is found in all multi-cellular organisms, including plants. This system is characterised by the presence of special molecules (termed receptors) on the cell surface of blood cells that have the ability to recognise pathogens. These receptors recognise the shape or pattern of molecules on the pathogen (PAMPS), which are often repetitive and carbohydrate (sugar) in nature. Thus carbohydrates play an important role in the recognition of pathogens by the immune system. Furthermore, in the change to malignancy the type and length of the sugars carried on cell surface molecules is often changed making the cancer-associated molecules distinct from those expressed by normal cells, allowing the cancer cells to be recognised by the immune system.

Each of the three events in this series of meetings was divided into sessions covering topical aspects of the subject and as well as presentations by invited speakers, most of the sessions contained talks chosen from submitted abstracts and given by young researchers.

1. Report on first event

**Carbohydrates and the Immune Response. Lesbos, Greece**

**28th May - 1st June 1999**

Sessions on carbohydrate receptors and antigen presenting cells, pathogens and carbohydrates, carbohydrates and the T cell and B cell response, glycosylation in mammalian cells and vaccine formulations were included.

A number of receptors on antigen presenting cells were described and these included the C-type lectin on macrophages described by Tatsuro Irimura and the mannose receptor described by Luisa Martinez-Pomares. The C-type lectin can bind glycosylated MUC2 peptides and the mannose receptor has been shown to have a domain, independent of its carbohydrate domain, which can bind to sialoadhesin. Interestingly, it has been previously shown that this molecule can bind to the MUC1 mucin.

The influence of the carbohydrates on the immune response to mucins was a recurring theme throughout the meeting and the enzymes involved in mucin type O-linked glycosylation were described by Henrik Clausen. Fredrik Olsen described a transient change in the glycosylation of MUC2 in response to infection and another member of the same group described the changes in intestinal mucin carbohydrates that occur in the mouse model of cystic fibrosis. The interaction of antibodies with MUC1 glycopeptides was described by Franz-Georg Hanisch who also reported that at least in a breast cancer cell line, there were differences in the amino acid sequence of some of the tandem repeats of MUC1. This was the first time that such an observation had been reported. The crystal structure of an antibody, reactive with a tumour associated epitope on MUC1, in complex with the glycosylated peptide antigen was also reported (Paul Freemont).

T cell responses to glycolipids and glycopeptides were reported by a number of participants. Stephen Porcelli gave an excellent presentation describing the presentation of lipids and glycolipids to T cells via CD1 molecules on antigen presenting cells. His group has identified several mycobacterial cell wall lipids that can be presented via CD1 molecules and have shown that isoprenoid phosphoglycolipids can be presented via CD1c. The work suggests that various CD1 isoforms have evolved to present different lipid antigens processed in different cellular compartments. Jeis Jensen and John Haurum presented their work on the presentation of glycopeptides to T cells via Class II and Class I and Lukas Heukamp presented a proffered paper that suggested that a H2-Kb restricted mouse CTL epitope found in MUC1 may be post-translationally modified, perhaps by glycosylation. The London group also reported on their work looking at human T cell responses to glycopeptides.

A number of presentations described the immune responses to lipopolysaccharides (LPS) which are found on Gram-negative bacteria, these included the possible use of the inner core of LPS as a vaccine against Neisseria meningitidis Group B. Further presentations on vaccine development were given by Philippe Moingeon, who described new approaches based on biological adjuvants, and by Ken Lloyd who discussed carbohydrate-based cancer vaccines. Hakan Steiner described the recognition of peptidoglycan, which is a fundamental component of bacterial cell walls and a candidate for a pattern to be recognised by the innate immune system. A protein (peptidoglycan recognition protein, PGRP) that has the ability to recognise this component has been cloned from insects. The group has also been able to clone a homologous protein from mouse and man and conclude that PGRP is a ubiquitous protein, conserved from insects to man, involved in innate immunity.
2. Report on second event

Mucins in Health and Disease. (6th International Workshop on Carcinoma-Associated Mucins). Robinson College, Cambridge, UK

29th July - 2nd August 2000

The meeting contained sessions on the expression of extracellular mucins, membrane mucins, sites and synthesis of O-glycans, glycosyltransferases, carbohydrate-protein interactions, mucin glycoproteins and disease, mucin glycoproteins and the immune response and clinical applications.

Extracellular mucins form large oligomers and the assembly of these oligomers was discussed by Juan Perez-Vilar and Ingemar Carstedt. Within this session the regulation of expression of two extracellular mucins, MUC2 and MUC5AC was also discussed.

Until two years ago when data was presented on MUC4 at the 5th Workshop, MUC1 was thought to be the only epithelial transmembrane mucin. Now it is clear that four of the cloned epithelial mucins are membrane proteins and data was presented by Stephanie Williams at this Workshop on the cloning of a novel mucin that is also a bound to the membrane. Data was presented by Jean Paul Aubert that alternative splicing of MUC4 can generate a family of secreted and membrane-associated mucins. Two such variants, MUC4/Y and MUC4/X both lack the tandem repeat and appear similar to the splice variant of MUC1 described by Daniel Wreschner that also lacks tandem repeats. This session generated a lot of discussion concerning the mechanism involved in the release of MUC1 from the cell membrane.

As a prelude to the session on glycosylation, Tommy Nilsson of Heidelberg presented his work concerning the maturation of cisterna and the recycling of glycosyltransferases through the Golgi apparatus. Initiation of mucin-type glycosylation was discussed by Eric Bennett of Copenhagen who reported on the presence of lectin-like domains in one particular polypeptide GalNAc transferase, GalNAcT4. In an in vitro system, this glycosyltransferase allows all the sites within the MUC1 tandem repeat to be substituted with O-glycans. Data showing the over-expression of a sialyltransferase, ST3Gal-I, in breast carcinomas which results in premature sialylation of sugar side-chains were presented by Martin Dalziel of London. This phenomenon explains at least in part the aberrant glycosylation of O-linked mucins observed in breast cancer.

The session on protein carbohydrate interactions was well received with Rodger McEver and Nobuyoshi Hiraoka discussing the non-epithelial mucin-like molecules which act as ligands for selectins. Paul Crocker from Dundee reported his work on the sialic acid binding proteins, the Siglecs with particular reference to their presence on cells of the innate immune system.

Mucin glycoproteins and the immune response was an extremely interesting session and Sandra Gendler (USA) reported her working showing that immune function is severely compromised in Muc1 knock out mice which was completely unexpected. Within this session the expression of MUC1 on cells of the haemopoietic system was reported by Mike McGuckin (Australia) and Aurelia Rughetti (Rome). This led into the Clinical Applications session where a clinical trial of vaccinia MUC1 was reported by David Miles and Bruce Acres. This session generated a large amount of discussion and in fact one of the successes of the Workshop was the high level of discussion that the majority of the papers generated.

Although all the posters were on display throughout the Workshop, allowing viewing at breaks and after the formal sessions, there were also three formal sessions dedicated to poster presentations which resulted in a total of six hours. This, together with the large number of oral proffered papers presented gave a large number of young researchers the chance to present their data to an international audience well known in the field of mucin research.

3. Report on third event

Carbohydrates and the Immune Response. Lesbos, Greece

31st August – 2nd September 2001

The meeting included an introductory session on glycosylation in mammalian cells which was followed by sessions on glycosylation and immune function, lectin interactions and the immune system, innate immunity and vaccines and carbohydrate antigens.

The meeting started with a talk by Henrik Clausen from Copenhagen who gave an introduction to O-linked glycosylation and the enzymes responsible for the addition of the first sugar, the GalNAcT family of glycosyltransferases. This family consist of more than 11 members and is conserved throughout evolution from fly to man. Interestingly, it was reported that some GalNAcTs have been shown to have functional lectin domains that can specifically bind to GalNAc carrying peptides. Tommy Nilsson from Heidelberg completed the introductory talks by discussing the sorting of glycosyltransferases in the Golgi apparatus.

Dietmar Vestweber gave a interesting talk on a genetic condition known as leukocyte adhesion deficiency II (LADII). These patients do not express fucosylated glycans which leads to immunodeficiency caused by lack of
selectin ligands. His group have cloned the gene responsible for this defect and shown it to be a GDP-fucose transporter. The selectins and their ligands is a very active area of research. Both E and P selectins are strongly expressed on endothelial cells undergoing cell division during angiogenesis in the tumour vasculature. John Magnani from the USA talked on the development of anatogonists of E and P selectins for use in targeting and killing vessels undergoing angiogenesis in tumours.

MUC1 was the first epithelial mucin to be cloned and has been studied extensively therefore there were a number of talks relating to this mucin. These included those discussing the changes in the glycans seen on MUC1 expressed by carcinomas (Joy Burchell, London and Stefen Mueller, Cologne), induction of humoral responses to MUC1 in MUC1 transgenic mice (Celso Reis, Porto) and the expression of MUC1 on activated T cells (Joyce Taylor-Papadimitriou, London). Peptides and glycopeptides based on the MUC1 tandem repeat sequence were also used a model for looking at the specificity of GalNAcTs (Henrik Clausen, Copenhagen), and the ability of dendritic cells to process glycopeptides (Olivera Finn, USA). Continuing the dendritic cell theme, lectin-like receptors present on dendritic cells were described by Tasturo Irimura from Japan and Yvette van Kooyk from the Netherlands presented her work on DC-SIGN. The excellent presentation given by Peter Beverley (Oxford) reported his work that showed that T cell immune responses are heavily influenced by microbial stimulation of dendritic cells.

Stephen Porcelli gave an excellent presentation updating the data that he presented at the first meeting. His group have recently identified a family of isoprenoid phosphoglycolipids that can be presented by CD1 that are broadly distributed among both prokaryotes and eukaryotes. The work suggests that, as well as their function in the recognition of pathogenic microbes, CD1 restricted T cells play a role in the regulation of the immune response and the prevention of autoimmunity.

4. Final assessment

Scientific

As stated in the introduction, the scientific importance of these meetings is evident by their continued success and the willingness of participants not eligible to be funded from the grant to be willing to fund themselves.

At each meeting new and exciting scientific developments have been presented and these are described above.

Impact of the project on participants.

As the Cambridge Mucin Workshops are the premier meetings in the mucin field they are the arena for the presentation of new results and the main venue for the formation of collaborative projects. The importance of the meetings to the delegates is testified by their continued return to the workshop and the willingness of many of the invited participants who are not eligible for EU funding, to finance themselves.

The series of meetings has allowed young European scientists to be involved in international workshops and to meet scientists who have an international reputation in the field.

As a result of these meetings interactions have been enhanced between several groups in Europe. Programmes involving researchers attending these conferences have been developed and several of these have been funded by the EU. The programmes for which we have details were co-ordinated by the organisers of these conferences. These are a Concerted Action Grant (contract number BMH1-CT94-1462) and RTD grants (contract numbers BI04-CT96-0139, QLK3-1999-00217) and two applications are pending. These projects have brought together scientists interested in the use of formulations based on MUC1 for immunotherapy of cancer and are composed of multidisciplinary groups and include a SME Biotechnology Company.

Future Meetings

The continued success of the Mucin Meetings has led us to the conclusion that they must continue. We have submitted an application to the EU for funding under the High Level Scientific Meetings Programme and a successful outcome will allow these meetings to stay in Europe till at least 2005. The proposed programme of events are as follows:

The 7th Symposium on Mucins in Health and Disease, 2-6th April, 2003 Crete, Greece
The 3rd Workshop on Carbohydrates and the Immune Response, late August, 2004, Lesbos, Greece
The 8th Symposium on Mucins in Health and Disease, July 2005, Cambridge, UK

Organisational

All three meetings were organised by Prof. Joyce Taylor-Papadimitriou and Dr Joy Burchell. The secretariat was Ms Gursharn Nutley. The advertising and the administration of the grants within the different categories are described in the reports for the individual events.
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PROGRAMME OF EVENTS

Event n°1 :
Carbohydrates and the Immune Response.
Lesbos, Greece
28th May - 1st June 1999

Event n°2 :
Mucins in Health and Disease. (6th International Workshop on Carcinoma-Associated Mucins.
Robinson College,
Cambridge, UK
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