Role of sugars in surface microbe–host interactions and immune reaction modulation

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What is known about the topic of this paper
• Sugars are vital components of both microbial and mammalian cells.
• They are involved in cellular communication and govern microbial virulence as well as host immunity and inflammation.
• The use of sugars to block microbial adherence and invasion to modulate inflammation offers great therapeutic potential.

What this paper adds to the field of veterinary dermatology
• This review summarizes current knowledge of carbohydrates and carbohydrate receptors relating to host–microbe interaction and immune reaction modulation in the skin.
• It identifies the therapeutic potential in controlling microbial disease that may be achieved by blocking microbial virulence mechanisms and modulating inflammation by means of simple and complex sugars.

Abstract
Sugars in the form of monosaccharides, oligosaccharides, polysaccharides and glycoconjugates (glycoproteins, glycolipids) are vital components of infecting microbes and host cells, and are involved in cell signalling associated with modulation of inflammation in all integumental structures. Indeed, sugars are the molecules most commonly involved in cell recognition and communication. In skin, they are essential to epidermal development and homeostasis. They play important roles in microbial adherence, colonization and biofilm formation, and in virulence. Two groups of pathogen recognition receptors, C-type lectins (CTL) and their receptors (CTLR), and the Toll-like receptors enable the host to recognize pathogen-associated molecular patterns (PAMPs), which are mainly glycolipids. The CTLs can recognize a wide variety of bacteria, fungi and parasites and are important in phagocytosis and endocytosis. TLRs are expressed on the surfaces of a variety of cells, including keratinocytes, dendritic cells, monocytes and macrophages; they play a major role in innate immunity. Interaction of TLRs with PAMPs initiates a cascade of events leading to production of reactive oxygen intermediates, cytokines and chemokines, and promotes inflammation. Exogenous sugars can block carbohydrate receptors and competitively displace bacteria from attachment to cells, including keratinocytes. Thus sugars may provide valuable adjunctive anti-inflammatory and/or antimicrobial treatment. A promising approach is the use of a panel of carbohydrate derivatives with anti-adhesive efficacy against bacteria frequently involved in diseases affecting skin and other epithelia. More complete characterization of sugar receptors and their ligands will provide further keys to use of carbohydrates in immunomodulation and infection control in skin.

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Introduction
The integument provides the essential barrier, which maintains homeostasis in animals and enables effective defence against microbial infection. The primary defensive capacity of the integument is provided by the epithelium of the respiratory, gastrointestinal and urinogenital tracts and, in skin, by the epidermis. The protective function of the epidermis is due to an integrated array of defence mechanisms incorporating the physical and chemical properties of the stratum corneum, its self-cleaning ability through desquamation, the secretions of the cutaneous glands and substances passing through the epidermis from the dermis, and the release from cells within the epidermis of peptides and lipid metabolites which are antimicrobial and may also inhibit microbial adherence. Thus, although the skin is constantly exposed to potential pathogens, such as Staphylococcus intermedius and Malassezia pachydermatis infection only occurs when the normal epidermal defences are disrupted.

In such circumstances, superficial infections may occur as a consequence of microbial adherence, proliferation and the production of virulence factors. In addition, biofilm production by certain organisms may confer special properties on the constituent microbes including enhancement of virulence and resistance to antimicrobial agents. Biofilms are created when microbes proliferate to form dense interacting communities on a solid surface, typically surrounded by an extracellular polysaccharide slime matrix. They are dependent on adherence to substrate, commonly host cells or inert substances such as catheters, the
availability of appropriate nutrients, including those released by inflamed and damaged host cells, and quorum sensing by the microbes.\textsuperscript{6–8}

Sugars and sugar receptors are vital components of the cellular structures of both infecting microbes and of the host cells.\textsuperscript{9,10} They are involved in microbial adherence, microbial uptake and in cell signalling associated with modulation of the inflammatory mechanisms in skin. In the epidermis, modulation of carbohydrate expression occurs during keratinocyte differentiation, and epidermal carbohydrates are involved in lipid maturation and the development of the epidermal barrier.\textsuperscript{11–13} Sugars are also involved in protection of the stratum corneum from proteolytic degradation\textsuperscript{14} and modification of sugar moieties may be important in epidermal homeostasis, barrier dynamics and desquamation.\textsuperscript{15,16} Cell surface glycoproteins are also involved in intercellular interaction between keratinocytes and dendritic cells.\textsuperscript{17}

Thus sugars have the potential to interfere with adherence and biofilm formation, and modulate surface barrier function and inflammatory reactions by the host. This review examines data supporting the use of simple and complex sugars in the control of cutaneous infections and inflammatory disease.

**Sugars in cell signalling and host–microbial interaction**

The sugars can be divided into two major subfamilies, the simple sugars (monosaccharides) and the complex sugars (oligosaccharides composed of two to 10 and polysaccharides with more than 10 monosaccharide molecules). The complex sugars link with proteins and fats to form glycoproteins and glycolipids (glycoconjugates) with a very broad range of properties. Indeed, sugars are the molecules most commonly involved in cell recognition and communication. They do this by means of lectins, ubiquitous complex proteins found in all living organisms that are able to recognize in a specific and reversible manner carbohydrates, such as D-galactose, D-mannose, D-fucose, sialic acid and N-acetyl-galactosamine that are exposed on cell membranes in the form of glycoproteins, glycolipids or polysaccharides (Fig. 1). They may be expressed by both host cells (endogenous lectins) and pathogens (exogenous lectins), and their expression varies according to the activity, maturity and differentiation of the cells, and on the status of cell surface glycoproteins. Thus surface glycoproteins are important in regulating and promoting cellular biological and immunological activity.\textsuperscript{18} However, lectins do not belong to the immune system, do not catalyse reactions and their structures remain poorly characterized.

The interaction between microbial agents and the animal host cell is multivalent. The abundance of carbohydrates in various forms at the animal cell surface is one reason why microbes to a large extent have evolved with the ability to adhere to sugar receptors in an organ-specific manner for colonization and infection.\textsuperscript{19}

Microbial lectins are glycoproteins that are known to be important virulence factors involved in specific interactions with host carbohydrate cell membrane receptors. They can interact with corresponding sugar moieties located on cell surfaces and may be blocked by exogenous carbohydrates.\textsuperscript{20} Carbohydrates at bacterial cell surfaces can also be involved in induction of virulence by promoting intercellular adhesion among microbial cells during biofilm formation (Figs 2 and 3).\textsuperscript{22–24}

Adherence, the specific attachment of microorganisms to epithelial cells (Figs 4–6), is an initial step in the pathogenesis of epithelial tissue infection. Biofilm formation and the production of capsular polysaccharide slime can also be important steps in this process and may be controlled by quorum sensing activity related to cell density.\textsuperscript{25,26} Bacterial adherence involves different types of lectins (adhesins), depending on bacterial strain and host cell. In vitro models thus require appropriately differentiated cells in order to study pathogen adherence. In the epidermis, pathogenic bacteria and fungi will bind to corneocytes,\textsuperscript{27–29} and any alteration of the keratinocyte differentiation process (e.g. wound healing, inflammation, and defective permeability barrier) can promote bacterial adherence.

There is evidence that carbohydrates are important in the pathogenesis of atopic dermatitis and associated microbial infections. In atopic dermatitis, decreased stratum corneum ceramide content may cause a defect in the permeability barrier function that helps initiate immunological reactions.
reactions and inflammation. Pathogenic staphylococci can colonize the skin of patients suffering from atopic dermatitis, and pathogenic staphylococci adhere more readily to corneocytes of atopic humans and dogs than to corneocytes of normal individuals or to dogs with primary

Figure 2. Diagrammatic representation of a bacterial cell. Adhesins enable attachment to host cells; they may form part of the cell wall or cell membrane, or be located on fimbriae and pili; they attach to glycoconjugates on host tissue such as fibrin and fibronectin. Capsular polysaccharide promotes adherence to host tissues and inert objects, e.g. catheters, and is involved in biofilm formation; it gives protection against phagocytosis. Cell wall protein A binds the Fc portion of immunoglobulin inhibiting phagocytosis. Cell wall, red; cytoplasm, yellow.

Figure 3. Diagram of the cell wall of Pseudomonas aeruginosa showing the location of capsular slime and the superficial lipopolysaccharide attached to the outer membrane. The anionic polysaccharide slime forms the matrix in which the cells become embedded during biofilm formation, and is able to bind cationic antibiotics and restrict their penetration. The lipopolysaccharide may also act as a permeability barrier to antimicrobials. After Lambert.21

Figure 4. Malassezia pachydermatis cells attached to canine corneocytes held on adhesive tape in an adherence study (crystal violet stain; image courtesy of Dr R. Bond).

Figure 5. Staphylococcus intermedius cells attached to canine corneocytes held on adhesive tape in an adherence study (crystal violet stain; image courtesy of Dr L. Saijonmaa-Koulumies).

Figure 6. Clusters of Malassezia pachydermatis cells in a clinical tape strip specimen from canine skin with Malassezia overgrowth. DiffQuik stain (image courtesy of Dr M-C Cadiergues).
seborrhoea. In man, one factor in this process is the redistribution of the glycoprotein, fibronectin, to the cornified layer in atopic dermatitis. It is also suggested that peptidoglycan of Staphylococcus aureus directly exacerbates inflammation in human atopic dermatitis. In dogs, the adherence in vitro to canine corneocytes of certain strains of *M. pachydermatis* and *S. intermedius* has been shown to be associated with mannose-bearing carbohydrate residues and with trypsin-sensitive proteins or glycoproteins.

Individual-specific sugars involved in adhesion of bacteria to a number of epithelial tissues were predicted and identified some time ago. Furthermore, several studies suggest that occupation of lectins on the bacterial surface by exogenous sugars can prevent bacterial adherence to epithelial cells of different tissues. Thus, the use of sugars to competitively displace bacteria from their attachment sites on cells would be particularly valuable when there is antimicrobial resistance. Mannose and N-acetyl-D-galactosamine inhibit the adherence of *Escherichia coli* and *Pseudomonas aeruginosa* to epithelial cells. A combination of D-galactose, L-mannose and L-rhamnose was found to decrease the adherence of *P. aeruginosa* to canine corneocytes by roughly 50% and to canine corneocytes by 48%.

Pathogen recognition receptors

Complex oligosaccharide structures displayed at microbial and host cell surfaces, incorporated into the extracellular matrix and attached to secreted glycoproteins can serve structural roles, mediate movement of glycoconjugates to the cell surface or act as markers that mediate cell–cell and cell–matrix recognition events. The nonstructural roles of sugars generally require the participation of sugar-binding lectins. Two groups of pathogen recognition receptors (PRR), the C-type lectins (CTL) and C-type lectin receptors (CTLR), and the Toll-like receptors (TLR), enable the host to recognize pathogen-associated molecular patterns (PAMP), mainly glycolipid structures. Lectins are often complex, multidomain proteins but their sugar-binding activity can usually be ascribed to a single protein module within the lectin polypeptide. Such a module is designated a carbohydrate-recognition domain (CRD). C-type lectins and CTLRs contain various CRDs and represent a very heterogeneous group with members such as the macrophage mannose receptor (MMR, Fig. 1) and langerin (see http://www.imperial.ac.uk/research/animallectins/). In recent years, a variety of these CTLs and CTLRs, such as dectin-1, DC-SIGN (CD209), and DEC-205 (CD205), have been intensively studied in the murine and human systems. The MMR is the best-characterized PRR to date. Initially identified in macrophages, it is involved in phagocytosis and endocytosis and can recognize mannose, fucose, glucose and N-acetyl-glucosamine, but not galactose, by means of a series of carbohydrate domains. It is able to recognize a wide range of bacteria, fungi and parasites through glycolipid PAMPs. It plays an important role in host defence against fungal pathogens and is involved in glycoprotein clearance. It is also expressed by immature dendritic cells. Furthermore, a keratinocyte mannose receptor (KMR) has been recently described. It exhibits similarities but also some differences from the MMR. The KMR has a different molecular mass and does not exhibit any cross-reactivity with monoclonal anti-MMR antibody. It resembles the MMR in that it is Ca²⁺-dependent and proteolysis sensitive, but it does not internalize mannose efficiently and is probably not a member of the endocytic CTLs. Langerin, another member of the CTLR family, is expressed with Birbeck granules, the specific intracellular organelles of Langerhans’ cells. Langerin (CD207) binds and mediates the uptake of sugar-containing molecules including mannose, fucose and N-acetyl-glucosamine. It serves to internalize microbial glycolipids into Birbeck granules where the glycolipids are loaded into CD1a for presentation to T cells.

So far, the only CTLR family members that have been identified in the canine system at the genomic level are CLEC2B and CLEC4F, and no functional data on these receptors have been published for the canine system. However, given their importance in pathogen neutralization and in organization of the extracellular matrix, the annotation of the canine genome will soon be followed by functional studies.

In addition to CTLR, and often forming complexes with them, TLRs do not seem to mediate the uptake of PAMPs but rather stimulate an intracellular signalling cascade. TLRs are expressed on the surfaces of a variety of cells, including epithelial cells, dendritic cells, monocytes and macrophages. They play a major role in innate immunity to microbial pathogens and are the subject of intensive study. Interaction of TLRs with their corresponding PAMPs initiates a rapid cascade of events leading to production of reactive oxygen intermediates, cytokines and chemokines, and promotes the inflammatory response. TLRs represent the main PRR for PAMP derived from gram-positive and gram-negative bacteria, as well as bacterial CpG motifs (unmethylated CG dinucleotide in specific base-sequence contexts). Sequences for canine TLRs 2, 4 and 9 have been published, and the presence of TLR transcripts has been investigated in different tissues. Canine peripheral blood neutrophils have been shown to respond to lipotechoic acid, a TLR2 ligand, with the production of interleukin (IL)-8 whereas this is not conclusive evidence that this reaction was TLR2-dependent, it clearly indicates that these cells are able to mount a pro-inflammatory response to a TLR2 ligand. Interestingly, keratinocytes have been reported to constitutively express TLR2, TLR3, TLR4, TLR5, TLR9 and to a lesser extent TLR10. This indicates that they are capable of responding to bacterial PAMPs, such as protein A of *S. aureus* which can cause serious damage to the skin. Injury to the skin, by either intrinsic factors (such as single nucleotide polymorphisms in the PRR genes) or extrinsic factors (such as disruption of the healthy skin integument), can impair the beneficial recognition of bacterial mono- and polysaccharides. Recent studies suggest that microbial components interact with signalling molecules of the TLR family to transduce signals in various cells, including keratinocytes. Treatment of keratinocytes with *Candida albicans*, mannan, lipopolysaccharide (LPS) and IFN-γ result in cell activation and increased IL-8 gene expression. This pathogen-induced expression of pro-inflammatory cytokine secretion is inhibited by anti-TLR2 and anti-TLR4 neutralizing antibodies.
suggesting that TLRs are involved in the signalling process. These findings open a new avenue to test carbohydrate molecules, either to stimulate the production of antimicrobial substances and/or to exert specific interference with TLRs.

**Immunomodulatory properties of monosaccharides**

Carbohydrate moieties are important sites of interaction for many lymphocyte functions including natural killer cell activity, lymphocyte–endothelial cell adhesion and lymphocyte recirculation. As long ago as 1984, Stankova and Rolapleszynski demonstrated that alpha-fucose inhibited human mixed lymphocyte culture reactions and subsequent suppressor cell generation. L-rhamnose has been shown to block splenocyte stimulation by LPS. Various sugars are also components of cytokine receptor sites, e.g. fucose and mannose, on the lymphocyte surface. These sugars can provide binding sites for extracellular lectins thus enabling them to play a role in cytokine-mediated signal transduction. Recent data indicate that some cytokines exhibit a carbohydrate recognition domain localized opposite the receptor binding domain, making cytokines bi-functional (Fig. 7). It is now recognized that several cytokines, including TNF and IL-1, have lectin-like activity. For instance, IL-1α and IL-1β exhibit distinct and specific carbohydrate-binding properties, which could explain why these two cytokines have different signalling profiles, and different target cells, despite sharing the same cell receptors.

Monosaccharides are able to inhibit the cutaneous delayed hypersensitivity reaction. Locally applied α-L-fucose inhibits the expression of delayed hypersensitivity response in a model of contact allergy sensitization and elicitation in mice. Application of α-L-fucose before sensitization has no suppressive effect, suggesting that the monosaccharide plays a role in the inflammatory phase only and that it acts locally. Recent data also argue for interesting pharmacological properties of L-fucose in wound healing and age-related modifications of dermal fibroblasts through collagen biosynthesis.

It is now recognized that keratinocytes play an important role in skin immunity by secreting a large number of cytokines. Keratinocytes express cytokine receptors on their surfaces, allowing them to respond to their own cytokine secretions (autocrine response) or to other soluble factors in their microenvironment. Activated keratinocytes are directly and indirectly responsible for the recruitment and local activation of leucocytes. Thus an intricate, cytokine-based signalling network in the epidermis can form the basis of a cascading inflammatory response in diverse skin diseases. Keratinocytes also possess the ability to synthesize and express cell surface moieties characteristic of effector or accessory cells of the immune system, and there is increasing evidence of the ability of sugars to influence cell-to-cell communication among keratinocytes and immune system activity.

Thus sugars prepared from purified Aloe polysaccharides reduce the amount of IL-10 observed in ultraviolet-irradiated murine keratinocytes. During the inflammatory response, keratinocytes can be induced to express the cell surface adhesion molecule ICAM-1/CD54 that binds to its ligand LFA-1 expressed on T cells, contributing to T-cell epidermotropism. Specific monosaccharides (fucose, rhamnose), alone or in combination, can reduce IFN-γ-induced ICAM-1/CD54 expression on human keratinocytes and down-regulate the secretion of proinflammatory cytokines IL-8 and TNF-α by activated keratinocytes (unpublished results). Masking of the cell membrane antigen or cell surface receptor by the sugar treatment is a likely explanation for these results.

Keratinocytes also have the ability to kill pathogenic fungi and bacteria by producing a large panel of antimicrobial substances. Some of these act by binding to microbial carbohydrates.

**Conclusions and perspectives**

The plasma membrane of mammalian cells contains numerous microdomains that are essential for cellular function and are biochemically composed of glycoproteins and glycolipids, which play a major role in the recruitment and concentration of molecules involved in cellular signalling.
and in acquired immune responses. Sugar receptors, when engaged by microorganisms or particles, represent signal transducing receptors that can trigger a variety of responses including secretion of mediators, cytokine production, and modulation of other cell surface receptors leading to inflammatory reactions. Conversely, the ability of exogenous sugars to block these specific receptors or/and to competitively displace bacteria from their attachment sites on cells may provide an adjunctive anti-inflammatory and/or antimicrobial treatment. A promising approach in cutaneous/epithelial superinfections is the use of a panel of carbohydrate derivatives displaying anti-adhesive efficacy against bacteria frequently involved in a number of diseases affecting the skin and other epithelia.

It would be also of major interest to develop molecules able to mimic commensal bacterial cell membrane glycoproteins. These commensals are regarded as beneficial for the host and provide protection by chronically stimulating epithelial surfaces to express antimicrobial peptides at levels that kill pathogenic microbes. Such molecules would specifically stimulate natural defence mechanisms that regulate the presence of bacteria on skin and epithelial surfaces, as well as exerting a protective immunomodulating effect on epidermal cells.

More complete characterization of the structure and function of sugar receptors and their ligands in the skin and epithelium would provide a better knowledge of the relationships between innate and adaptive immunity and their interaction in defence reactions. Such knowledge will provide further keys to the use of carbohydrates in immunomodulation and infection control in skin.

References
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 sont exprimés à la surface de cellules variées, incluant les kératinocytes, les cellules dendritiques, les monocytes et les macrophages; ils jouent un rôle majeur dans l'immunité innée. L'interaction des TLRs avec les PAMPs provoque une cascade d'événements provoquant la production de réactifs oxygénés, de cytokines et de chémokines, et facilite l'inflammation. Les sucres exogènes peuvent bloquer les récepteurs et éliminer les bactéries des cellules, incluant les kératinocytes. Les sucres sont donc des traitements associés anti-inflammatoires et/ou antimicrobiens. Une approche intéressante est l'utilisation d'un ensemble de sucres avec une action antiadhésive sur les bactéries les plus souvent impliquées dans les maladies cutanées. La détermination plus précise des récepteurs aux sucres et de leurs ligands permettra des solutions futures pour l'utilisation des sucres dans l’immunomodulation et le contrôle de l’infection cutanée.

Resumen  Los azúcares en la forma de monosacáridos, oligosacáridos, polisacáridos y glicoconjugados (glicoproteínas, glicolípidos) son componentes vitales de los microorganismos infecciosos y las células hospedadoras, y están implicados en las señales celulares asociadas con la modulación de la inflamación en todas las estructuras tegumentarias. De hecho los azúcares son las moléculas más implicadas en el reconocimiento y comunicación celulares. En la piel son esenciales para el desarrollo y la homeostasis. Juegan un papel importante en la adherencia microbiana, colonización y formación de películas biológicas (biofilms) y virulencia. Dos grupos de receptores de reconocimiento de patógenos, lectinas de tipo C (CTL) y sus receptores (CTLR), y los receptores tipo TOLL (TLRs), permiten al hospedador reconocer patrones moleculares asociados a agentes patógenos (PAMPs), que son principalmente glicolípidos. Los CTL pueden reconocer una amplia variedad de bacterias, hongos y parásitos, y son importantes en la fagocitosis y endocitosis. Los TLRs son expresadas en la superficie de una gran variedad de células, incluidos queratinocitos, células dendríticas, monocitos y macrófagos, y juega un papel importante en la inmunidad innata. La interacción de los TLRs con los PAMPs inicia una cascada de procesos que llevan a la producción de intermediarios reactivos de oxígeno, citocinas y quimoquinas y promueven la inflamación. Los azúcares exógenos pueden bloquear los receptores de carbohidratos y desplazar de forma competitiva bacterias de su unión con las células, incluidos los queratinocitos. Así pues los azúcares pueden proporcionar una ayuda valiosa en el tratamiento antiinflamatorio y antimicrobiano. Una estrategia prometedora es el uso de un grupo de derivados de carbohidratos con propiedades antiadhesivas frente a bacterias implicadas en enfermedades de la piel y otros epitelios. Una caracterización mas completa de los receptores de azúcares y sus ligandos proporcionará mas claves en el uso de carbohidratos para el control de la inmunomodulación e infección en la piel.