Multi-author Review
Lactoferrin

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Cell. Mol. Life Sci. 62 (2005) 2529–2530
1420-682X/05/222529-02
DOI 10.1007/s00018-005-5366-y

Cellular and Molecular Life Sciences

Lactoferrin: A multi-tasking protein par excellence

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Online First 2 November 2005

Since its first identification as a 'red protein' in bovine milk more than 65 years ago [1], and its purification in 1960 from human and bovine milks [2–4], lactoferrin has intrigued and puzzled researchers. Central to the debate has been the question of its 'real' function. Its iron binding properties, and its presence in milk in predominantly iron-free form, led to an initial presumption that its function was protective, but might also serve a nutritional role for the infant. Some properties that derived from this – for example, its ability to inhibit bacterial growth through iron deprivation [5] – were quickly established, but it has remained frustratingly difficult to determine whether lactoferrin also has a role in iron absorption. Subsequent determination of its amino acid sequence [6], three-dimensional structure [7] and detailed iron binding properties firmly established lactoferrin as a member of the transferrin family, and reinforced the natural presumption that its biological function related to iron binding. Since then, an overwhelming weight of evidence has pointed to a much more complex situation.

The current view of lactoferrin challenges the paradigm of –'one protein, one function'. A short list of demonstrated activities would include anti-bacterial, anti-viral and anti-tumour activities; regulation of cell growth and differentiation; anti-inflammatory activities; and modulation of the immune response. Every year or two, a new and surprising function pops up – for example, its proteolytic activity [8], its inhibition of bacterial biofilm [9] and its promotion of bone growth [10]. Many of its activities can be grouped under the heading of host defence, but it is clear that no single mechanism applies. Nor is it known whether the same properties are shared by all lactoferrins, or whether some are particular adaptations, or even 'accidental' activities in a particular host. Whatever the truth, lactoferrin certainly offers real potential as a therapeutic agent, providing the mechanism for a particular activity can be unravelled, and the required concentrations for use can be established. In this collection of reviews, we set out to examine the current state of knowledge across the whole range of biological activities expressed by this fascinating protein.

The first two reviews provide overviews of lactoferrin structure and function. In the first, focusing on three-dimensional structure, the relationship between structure and metal binding is set out. Both folding and dynamics play a critical role, as domains close over the bound metal ions to sequester them or move apart to release them. These principles seem to apply the binding not only of Fe^{3+} ions, but also of other metal ions as well, giving lactoferrin a potentially wider role in metal ion homeostasis. The protein surface is clearly critical to many of the other biological activities of lactoferrin. Although there is one very prominent region of positive charge which appears to be largely conserved, amino acid variations on the surface are likely to result in real differences in its specificity for target molecules on cells, emphasizing the need for caution in extrapolating functional conclusions from one lactoferrin to another. In the second review, Ward et al. provide a comprehensive functional overview. The important issue of iron homeostasis is discussed in the light of a mouse model of lactoferrin deficiency [11] which argues against a direct role in iron absorption and in favour of a broad defence role. An emerging theme is the ability of lactoferrin to regulate cellular signalling pathways, which affect activities such as its alleviation of inflammation, promotion of bone growth and suppression of carcinogenesis. Thus its anti-inflammatory activity is linked to an ability to inhibit the production of pro-inflammatory cytokines, but by several distinct mechanisms, and its regulation of bone growth apparently occurs through mitogen-activated protein (MAP) kinase pathways. Likewise, increasing numbers of studies show that lactoferrin possesses anti-cancer properties that apparently stem from its ability to modulate pathways that impinge on the cell cycle or result in upregulation of the expression of cytokines such as interleukin-18.

The theme of regulation of signalling pathways is taken up in more detail by Legrand et al. in the third review. This review focuses on the ability of lactoferrin to

modulate the immune and inflammatory response. The presence of lactoferrin in the secondary granules of neutrophils [12] has always hinted at a role in immune modulation, and an impressive array of activities is now documented. Binding to immune cells enables lactoferrin to play a role in lymphocyte maturation and activation, and it has increasingly clear roles in regulating the expression of cytokines, impinging on the inflammatory and immune responses. The authors emphasize that lactoferrin can bind to a wide variety of cells, either through specific receptors or through its ability to bind to glycosaminoglycans, and can also bind bacterial lipopolysaccharide. This means that it can act through a variety of mechanisms which can be hard to disentangle. The regulation of its synthesis, secretion and presence at particular sites, and under particular conditions, is also critical. The fourth review, by Suzuki et al., picks up on the key issue of receptor binding, and is of great importance in describing the first fully-characterized lactoferrin receptor (LfR), the LfR from human small intestine [13]. This receptor, also known as intelectin, had previously been isolated from mice, and is now shown to be widely expressed in numerous mammalian tissues. The possible roles of this receptor in iron absorption and in regulating cellular signalling pathways are discussed, with the implication that this key advance will now enable the elucidation of molecular mechanisms for some of these activities. The interaction of lactoferrin with other receptors, such as the liver lipoprotein receptor-related protein (LRP) and asialoglycoprotein (ASGP) receptors, lymphocyte and monocyte receptors, is also described, but fuller molecular characterization of these receptors remains a high priority.

Finally, the reviews by Valenti and Antonini, and by Gifford et al., discuss two new directions that have emerged since the original findings of bacteriostatic activity by lactoferrin. In the first of these reviews, Valenti and Antonini show that bacteriostatic activity, through iron deprivation, was only the tip of an anti-microbial iceberg. Many new mechanisms have emerged through which lactoferrin expresses anti-bacterial properties, including direct bactericidal action that results from binding to and disruption of bacterial cell walls; proteolysis of bacterial virulence factors; disruption of bacterial adhesion; and inhibition of biofilm formation. A striking range of antiviral activities against a number of very important human viruses has also been discovered. The authors warn that care must be taken in extrapolating results, in view of the different environments and microbial and viral responses that apply. The weight of evidence makes it clear, however, that lactoferrin is a key host defence factor; indeed, its presence in mucosal secretions identifies it as in the front line against attack by a wide variety of pathogens. The final review, by Gifford et al., concerns one of the most surprising and important discoveries to have come from research on lactoferrin. The discovery of a domain in the N-terminal region of the protein, which could be liberated in vivo by proteolysis, and which had potent bactericidal properties in its own right [14], has generated huge interest. Two versions of the peptide, lactoferricin H (LfcinH, derived from human lactoferrin) and lactoferricin B (LfcinB, from bovine lactoferrin) have drawn most attention, but variants have also been investigated. Gifford et al. review the structures assumed by these peptides, the mechanism through which they pass through the bacterial cell wall and enter the cytoplasm, and the most important determinants of Lfcin action. While the mode of action of Lfcin, once inside the cell, is still uncertain, there is now the possibility of using quantitative structure-activity relationship analysis (QSAR) to design more potent peptides for therapy. From the point of view of lactoferrin it is intriguing to think that one of its roles may be to sacrifice a portion of its polypeptide to generate a more potent anti-bacterial weapon.

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