Advanced Drug Delivery Reviews 64 (2012) 1759–1781

Contents lists available at SciVerse ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

On the roles of polyvalent binding in immune recognition: Perspectives in the nanoscience of immunology and the immune response to nanomedicines ☆

Thomas Vorup-Jensen *

Biophysical Immunology Laboratory, Department of Biomedicine, Aarhus University, Aarhus, Denmark
The Lundbeck Foundation Nanomedicine Center for Individualized Management of Tissue Damage and Regeneration, Aarhus University, Aarhus, Denmark
Interdisciplinary Nanoscience Center, Aarhus University, Aarhus, Denmark

ARTICLE INFO

Article history:
Received 8 April 2012
Accepted 8 June 2012
Available online 15 June 2012

Keywords:
Polyvalent binding
Avidity
Adhesion molecules
Integrins
Immunoglobulins
Mannan-binding lectin
Nanotoxicology

ABSTRACT

Immunology often conveys the image of large molecules, either in the soluble state or in the membrane of leukocytes, forming multiple contacts with a target for actions of the immune system. Avidity names the ability of a polyvalent molecule to form multiple connections of the same kind with ligands tethered to the same surface. Polyvalent interactions are vastly stronger than their monovalent equivalent. In the present review, the functional consequences of polyvalent interactions are explored in a perspective of recent theoretical advances in understanding the thermodynamics of such binding. From insights on the structural biology of soluble pattern recognition molecules as well as adhesion molecules in the cell membranes or in their proteolytically shed form, this review documents the prominent role of polyvalent interactions in making the immune system a formidable barrier to microbial infection as well as constituting a significant challenge to the application of nanomedicines.

Contents

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "Nanotoxicity: from bench to bedsite".

* Corresponding author at: Department of Biomedicine, Aarhus University, The Bartholin Building (1240), Wilhelm Meyers Allé 4, DK-8000 Aarhus C, Denmark. Tel.: +45 8716 7853.
E-mail address: vorup-jensen@microbiology.au.dk.

0169-409X/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.addr.2012.06.003

1. Introduction

Any introduction to the field of immunology, at the novice or expert level, is compelled at some point to convey the image of large molecules, either in the soluble state or in the membrane of some leukocyte, forming multiple contacts with a target for actions of the immune system. With the several functions of the immune system, targets include viral or bacterial microbes, cells of host tissue, the extracellular matrix (ECM)[1] surrounding these cells, or even engineered material such as drugs and drug delivery vehicles. In explaining the role of the multiplicity of bond formation between the immune cell or molecules and their targets, it has traditionally been the focus that polyvalency increases the binding strength in a non-linear, often *quasi* exponential, fashion with linear increments in valency. The phenomenon is so experimentally striking that it has received the name of avidity [1].

As reviewed in Section 1, inquiry into the relationship between the affinity of a monovalent interaction and the avidity of its polyvalent correspondent has been the subject of analyses for the better part of the past 100 years. Unlike many other topics in immunology, the list of early contributions cannot easily be dismissed as of only historical interest, since many important issues of understanding the basic chemistry of polyvalent interactions remain scarcely understood.

Section 2 addresses some of the roles of polyvalency in the interaction between receptors in the cell membrane and their ligands. Tethering of these receptors in the membrane is functionally significant with regard to supporting cell contacts as well as the far more complex issue of regulating intracellular and intercellular signaling of leukocytes.

Humoral factors of the immune system include plasma proteins such as the abundant immunoglobulins as well as other large proteins with the ability to activate the complement system. With the relatively fixed structure of these proteins, the topology of surface-presented epitopes or ligands on targeted surfaces vastly influences avidity. As discussed in Section 3, both steric and thermodynamical factors points to ultrastructural aspects as relevant in the distinction between surfaces targeted and non-targeted by the immune system. Receptor shedding creates intermediaries between cellular and humoral factors in the immune system. Also in this case, a significant aspect of the structural biology of these shed receptors is found in their ability to form oligomeric structures permitting polyvalent interactions with their ligands.

Finally, Section 4 discusses the role and nature of polyvalent interactions between molecules of the immune system and drug delivery vehicles and other nanostructured physical objects. The well-defined chemical nature of engineered nanoparticles facilitates a relatively rigorous approach to what features enables interactions with polyvalent molecules or cell membrane-bound receptors. It is proposed that a particular toxicological aspect of nanomedicine originates in the nm-scaled presentation of ligand for molecules of the immune system. This also opens a more general perspective on the nanostructured features that may elicit immune responses, extending to functions of the immune system in clearing microbial threats.

1.1. A brief history of avidity as a concept in immunology

As noted by others the concept of avidity has no easily identifiable origin [1]. For this reason it is helpful to present a brief historical perspective on the development of the concept.

In early stages of molecular immunology avidity was a loosely defined term to imply a semi-quantifiable binding between biological molecules, notably antibodies, as indicated, for instance, by the first report in *The Journal of Immunology* using the term avidity published in 1916 [2]. From the growing awareness of the large size of proteins and careful enumeration of the antigen binding strength from neutralization of viral infectivity, a likely first suggestion [3] of polyvalency as playing a role in immune recognition was made by Burnet et al. in 1937 [4]. These authors suggested that "an antibody molecule already held by one bond to the virus surface is brought into such a position that another linkage between an antigenic determinant and corresponding area on the antibody molecule becomes possible" [4]. Their report is remarkable, not only for use of the Langmuir equation [5] as a model for the antibody binding to antigens, but also in its foresight on the challenges ahead by pointing to three major aspects of immune protein recognition of targets, namely the surface presentation of these targets, the striking heterogeneity of the interactions as observable from simple titration of immune sera, and the role of polyvalency in explaining an apparent irreversibility of the binding reaction. All of these aspects prevail as current challenges in understanding the biochemistry of polyvalent binding as discussed in the present review. Even the sequential order of bond formation suggested by Burnet et al. [4] has remained the preferred reaction scheme for polyvalent interactions of antibodies [3,6,7]. The experimental demonstration of antibody polyvalency was made by Eisen & Karush in 1949, who showed that the predominant antibody in immune sera was bivalent, likely in these experiments to be IgG [8]. From the enzymatic tool box developed by Porter, which enabled the separation of the Fab and Fc fragments by proteolysis of intact antibodies [9], it became possible to detail the position of antibody combining sites within IgG. This work produced in 1963 the iconic "Y"-shaped structure ultrastructure of IgG [10], which left no doubts about the bivalency of the molecule.

The proteolytic separation of the Fab and Fc fragments [9] also enabled experiments to show that the avidity, or, with a term suggested by Karush, "functional affinity" [3], of the intact immunoglobulin was vastly larger than the affinity of the monovalent Fab [11]. To provide a theoretical rational for this finding, apparently some investigators chose to explain the enhancement of binding strength by tethering of Fabs as originating from the simple sum of Gibbs energies contributed through the epitope binding by each Fab [6,12]. Indeed, from the relationship in chemical reaction between standard change in Gibbs energy (ΔG^0) and the association equilibrium constant (K_A), *i.e.*, $\Delta G^0 = -RT \ln K_A$, where R is the gas constant and T the absolute temperature, it was proposed that the avidity of the interaction would grow exponentially with the number (n) of Fabs forming bonds to epitopes with the resulting association constant largely proportional to $(K_A)^n$. Echoing Burnet et al.'s awareness that polyvalent binding would require some degree of spatial order of the epitope presentation [4], limitations on the polyvalency-driven increment of binding strength was mainly claimed to be found in the stereochemistry of the interaction between the IgG molecule and epitope-presenting surfaces [6]. However, as discussed further below, a simple additivity of the Gibbs energies of the monovalent interactions as a description of the Gibbs energy of a polyvalent interaction was dismissed by Jencks as inappropriate from a theoretical point of view [12].

Over at least the past four decades several reports have regularly employed avidity as a term to describe the strength of cellular adhesion. As in the case of the initial use for characterizing antibodies, this was apparently meant to indicate a semi-quantitative phenomenon. However, an influential paper published by Bell in 1978 [13] focusing on specific adhesion of cells to cells points to another perspective. Bell was keen to stress that the biophysics of membrane-bound receptors

[1] Non-standard abbreviations used in this paper: AFM, atomic force microscopy; APC, antigen-presenting cell; CR, complent receptor; CRD, carbohydrate recognition domain; GA, glatiramer acetate; IC_{50}, 50%-inhibitory concentration; ICAM, intercellular adhesion molecule; IgSF, immunoglobulin super family; JAM, junctional adhesion molecule; LFA, lymphocyte function-associated antigen; LPS, lipopolysachharide; MBL, mannan-binding lectin; MHC, major histocompatibility complex; MIDAS, metal ion-dependent adhesion site; MMP, matrix-metalloproteinase; M_r, relative molecular mass; NSOM, near-field scanning optical microscopy; QSAR, quantitative structure-activity relationship; RGD, arginine-glycine-aspartate; RMSD, root-mean-square deviation; RU, resonance units; SAXS, small angle X-ray scattering; SLT, Shiga-like toxin; SPR, surface plasmon resonance; TLR, Toll-like receptor; VCAM, vascular cell adhesion molecule; VLA, very late antigen.