Current Topics in Microbiology and Immunology

205

Springer
Berlin
Heidelberg
New York
Barcelona
Budapest
Hong Kong
London
Milan
Paris
Santa Clara
Singapore
Tokyo

The CD4 Molecule

Roles in T Lymphocytes and in HIV Disease

Edited by D.R. Littman

With 29 Figures

Springer

Professor DAN R. LITTMAN, M.D., Ph.D.
Howard Hughes Medical Institute
Division of Molecular Pathogenesis
Skirball Institute of Biomolecular Medicine
New York University Medical Center
540 First Avenue
New York, NY 10016
USA

Cover illustrations: The HIV receptor. A. A ribbon diagram of domain 1 of human CD4 from the standard point of view (e.g., Figure 2 of Brady and Barclay, Chapter 1, this volume). The N-terminus of strand A and the C-terminus of strand G are marked. B. A space-filling model of domain 1 of human CD4 from the point of view of a monomer of gp120 approaching the contact site. In both panels, residues are colored similarly. Residues 86–89 (F-G turn, equivalent to DCR3 of Ig variable domains) are shown in green. Residues 33–62 (C'–C"–D, equivalent to CDR2) are shown in red, with the exception of the following. Residues Lys29, Lys35, Lys46 and Arg59 are shown, with their side-chains, in yellow. Residue Phe43, with its side-chain, is shown in off-white. The remaining residues of the C" ridge (Ser42, Leu44, Thr45, Gly47) are shown in magenta. All other residues are shown in blue.

Cover design: Künkel+Lopka, Ilvesheim

ISBN-13: 978-3-642-79800-9 e-ISBN-13: 978-3-642-79798-9
DOI: 10.1007/978-3-642-79798-9

Library of Congress Catalog Card Number 15-12910
Softcover reprint of the hardcover 1st edition 1996

Typesetting: Thomson Press (India) Ltd, Madras
SPIN: 10485113 27/3020/SPS – 5 4 3 2 1 0 – Printed on acid-free paper

Preface

During the late 1970's the application of hybridoma technology led to an explosion in the discovery and characterization of proteins expressed at the surface of hematopoietic cells. The understanding of T lymphocyte biology benefited enormously from this advance and from newly developed techniques for obtaining clonal T cells. Application of these methodologies resulted in the identification of the clonally restricted T cell antigen receptors (TCRs) and of a number of other molecules expressed more broadly on T cells. Among these, the CD4 and CD8 glycoproteins stood out because they were differentially expressed on distinct functional subsets of T lymphocytes. Moreover, blocking studies with monoclonal antibodies suggested a functional role for CD4 and CD8 in T cell responses to antigen. Shortly thereafter, it was shown that T helper cells were the primary targets for the human immunodeficiency virus (HIV) and that CD4 serves as the viral receptor on these cells. These findings fueled an intense interest in CD4 during the last decade, in the hope that understanding the molecular nature of the HIV-CD4 interaction could hold the key to controlling AIDS.

The interesting biological function of CD4 was gleaned early on from the observation that its expression is restricted primarily to T helper cells. The finding that the cognate molecule, CD8, is expressed exclusively on cytotoxic T cells and the subsequent recognition that $CD8^+$ cells interact specifically with MHC class I molecules suggested that these glycoproteins may interact directly with different classes of MHC proteins (SWAIN 1981). Subsequent studies showed that, indeed, CD4 and CD8 bind to regions of MHC class II and class I, respectively, that are distinct from the antigen-binding pockets recognized by the TCR (see Chap.2). Early work with blocking antibodies suggested that CD4 is involved in mediating adhesion, thus strengthening the interaction of "low affinity" TCRs with MHC/antigen (MARRACK et al. 1983). The subsequent discovery of the association of the protein tyrosine kinase $p56^{lck}$ with CD4 resulted in a turnaround in the view of CD4 function, with most investigators

favoring a signaling role via associated Lck after binding of CD4 to MHC (RUDD 1990). More recent studies suggest that both interpretations are partially correct (KILLEEN et al. 1993).

Cloning of the CD4 gene in the mid-1980's made it possible to examine the structural and functional features of CD4 in depth. These studies have defined the role of CD4 as a co-receptor (JANEWAY 1989) which participates in a complex with the TCR to recognize MHC/antigen and to initiate various signals in developing thymocytes or mature T cells. Genetic manipulation of the CD4 cDNA permitted demonstration of the direct interaction of the gene product with MHC class II. In addition, this allowed large quantities of protein to be produced for crystallization and structural studies. Introduction of mutant forms of CD4 into functional T lymphocytes further permitted dissection of the role of different domains of the molecule in the process of antigen recognition. The most recent studies have exploited gene targeting and transgenic technologies to explore the function of CD4 in the developing thymus.

Although initial attention to CD4 was due to the important role of the molecule in T lymphocyte activation and development, it was the discovery of the essential and complex role of CD4 in the life cycle of HIV that has contributed to much of the recent interest in the structure and function of the glycoprotein. In the mid-to-late 1980's, the terms "T4 cells" and "CD4 cells" made their entry into the popular lexicon, as awareness of the importance of these cells in the progression to AIDS began to reach the lay public. The reviews presented in this volume represent an attempt to provide a comprehensive overview of current research on the functions of CD4 in the normal immune response and in HIV disease. The subject areas have been divided into the following categories: structure and function of the CD4 molecule, particularly as it involves its interaction with MHC class II and the TCR complex (Chaps. 1,2); signaling functions of CD4 and the associated protein tyrosine kinase, $p56^{lck}$ (Chaps. 3,4); the role of the CD4 glycoprotein and of CD4 gene regulation in development (Chap. 5); the cell biology of CD4, including its intracellular interactions with HIV gene products (Chap.6); and the functions of CD4 in HIV entry and pathogenesis (Chaps. 7,8).

Some areas of research on CD4 are covered in greater depth than others, largely because more is known about them. For example, the crystal structures of the two component parts of the extracellular domain of CD4 have been elucidated. Much is also known of the interaction of CD4 with MHC class II and of its requirement for activation via the TCR complex. Recent

studies also provide a clearer understanding of the role of CD4 in the development of functional subsets of T lymphocytes. In HIV disease, the interaction of the viral receptor, CD4, with the viral envelope glycoprotein has been worked out in some detail (discussed in Chaps. 1,7), but an in vivo function for CD4 in virus-induced cell death remains a matter for much speculation (Chap. 8).

Several areas of CD4 biology are not covered in this monograph, but their importance should by no means be ignored. One of these, in which our understanding remains sketchy, is the phenomenon known as "negative signaling". This refers to inhibition of TCR-mediated signal transduction when there is prior engagement of CD4 with cross-linking antibodies. This phenomenon has been most clearly described using primary T lymphocytes. For example, cross-linking of CD4 upon binding of HIV envelope glycoprotein has been shown to result in increased apoptosis of human T cells, and has been suggested as a potential mechanism of T cell death in AIDS (see Chap. 8). The signaling processes resulting in signal abrogation or induction of apoptotic cell death have not been elucidated, but one study has suggested that sequestration of $p56^{lck}$ by CD4 from the TCR complex may contribute to this phenomenon (HAUGHN et al. 1992). There is also evidence that cross-linking of CD4 upon HIV binding results in NFkB translocation to the nucleus, where it would enhance viral gene expression (BENKIRANE et al. 1994). This result suggests a signaling role for CD4 that is independent of the TCR. However, this area is quite controversial, since another study provides evidence for an inhibitory rather than a stimulatory role for the CD4 cytoplasmic domain in viral replication (TREMBLAY et al. 1994).

Another area of interest, where a mechanistic understanding is lacking, is the phenomenon of T cell phenotypic switching observed upon treatment of animals with anti-CD4 antibodies. For example, in response to injection of *Leishmania major*, Balb/c mice mount a response that is dominated by antigen-specific T_h2 cells rather than protective T_h1 cells, and the animals succumb to the infection. If anti-CD4 is injected at the time of infection, a dominant Th1 response is mounted, and the animals are protected (SADICK et al. 1987). Treatment with nondepleting anti-CD4 antibodies has also been shown to result in immunological tolerance to various antigens that are administered concurrently. For example, some strains of mice will accept allogeneic skin grafts when anti-CD4 is administered for a brief period of time after grafting. The animals will then accept subsequent grafts from the same allogeneic source, but

will reject third party skin grafts. Most remarkably, $CD4^+$ cells from the tolerized mice can transfer skin graft tolerance to naive recipient mice that never received anti-CD4 treatment (Qin et al. 1993). In these transfers, the host T cells assume the tolerizing function, suggesting that the antigen specific tolerizing T cells, effectively suppressor cells, may shift the host T helper cell response from an inflammatory response to one that keeps the inflammatory T cells in check. The mechanisms through which anti-CD4 effects these apparent switches in T cell function in vivo are not understood. Nevertheless, a large number of studies using anti-CD4 therapy have been initiated. In mouse model systems for autoimmunity, such as NZB/NZW mice which develop a lupus-like disease, anti-CD4 has been shown to be quite effective (Wofsy and Carteron 1990). Based on these successes in the mouse, several trials of anti-CD4 therapy in human diseases, such as rheumatoid arthritis and psoriasis, are being conducted.

In the pathogenesis of AIDS, the CD4 molecule plays key roles, not only as receptor for HIV, but also as a target of interactions with the HIV gene products Nef and Vpu and, potentially, as a contributor to aberrant T cell activation resulting in premature T cell death. In humans, CD4 is expressed not only on T helper cells, but also on macrophage lineage cells, including microglial cells in the brain. Use of soluble forms of CD4 in vitro can effectively block infection with laboratory strains of HIV. However, primary strains of HIV are relatively resistant to this treatment, in large part due to low affinity of soluble CD4 for the oligomeric envelope glycoproteins of these virions. It is therefore not surprising that trials of soluble CD4 in AIDS patients have to date been unsuccessful. Recent studies suggest that infection of macrophages may be critical early in the course of the disease, raising the possibility that manipulation of CD4 on macrophages, where it is not thought to have an important physiological function, may attenuate viral spread in vivo.

Two of the "nonessential" HIV gene products, Nef and Vpu, have important interactions with CD4. These gene products are dispensable for viral replication in vitro. However, Nef, in the closely related simian immunodeficiency virus (SIV), is essential for induction of disease in infected rhesus macaques. Expression of Nef from HIV and SIV results in down-regulation of CD4 from the cell surface through a mechanism of enhanced endocytosis (see Chap. 6 for a more complete treatment of this subject). Nef, a myristylated membrane-associated protein of 27 kDa, also appears to affect signal transduction via the TCR

complex, possibly increasing sensitivity of the cell to antigenic stimulation. It is not yet known if CD4 down-regulation is essential for the in vivo effect of Nef or if the Nef effects on CD4 and on T cell signaling are causally related (LITTMAN 1994). The importance of Vpu in vivo is not yet known (Vpu is absent from SIV, so it is more difficult to study). Vpu is an integral membrane protein that enhances virus particle release and causes degradation of CD4 within the endoplasmic reticulum following an interaction mediated through the cytoplasmic domain of CD4. Degradation of CD4 is dependent on phosphorylation of two serines within Vpu (SCHUBERT and STREBEL 1994). Together, Nef and Vpu thus decrease the level of surface CD4 in HIV-infected cells; this may facilitate release of virus from the cell surface (by decreasing the possibility of virus binding to the cell from which it is released) or the enhanced activation of the T cell through indirect mechanisms, resulting in enhancement of viral transcription and production of infectious particles. Interruption of the interaction of the HIV gene products with CD4 may therefore have practical consequences in the control of HIV disease.

From this brief introduction, it should be clear that a comprehensive understanding of the interactions of CD4 with a number of extracellular and intracellular molecules is likely to contribute to the development of practical approaches for controlling normal and abnormal immune responses and the replication of HIV and its pathogenic consequences.

New York, USA DAN R. LITTMAN

References

Benkirane M, Jeang K T, and Devaux C (1994) The cytoplasmic domain of CD4 plays a critical role during the early stages of HIV infection in T-cells. EMBO J 13: 5559–5569

Haughn L, Gratton S, Caron L, Sekaly R P, Veillette A, Julius M (1992) Association of tyrosine kinase p56lck with CD4 inhibits the induction of growth through the alpha beta T-cell receptor. Nature 358: 328–331

Janeway C A Jr (1989) The role of CD4 in T-cell activation: accessory molecule or co-receptor? Immunol Today 10: 234–238

Killeen N, Davis C B, Chu K, Crooks M E, Sawada S, Scarborough J D, Boyd K A, Stuart S G, Xu H, Littman D R (1993) CD4 function in thymocyte differentiation and T cell activation. Phil Trans Royal Soc London 342: 25–34

Littman D R (1994) Immunodeficiency viruses. Not enough sans Nef. Curr Biol 4: 618–620

Marrack P, Endres R, Shimonkevitz R, Zlotnik A, Dialynas D, Fitch F, Kappler J (1983) The major histocompatibility complex-restricted antigen receptor on T cells. II. Role of the L3T4 product. J Exp Med 158: 1077–1091

Qin S, Cobbold S P, Pope H, Elliott J, Kioussis D, Davies J, Waldmann H (1993) "Infectious" transplantation tolerance. Science 259: 974–977

Rudd C E (1990) CD4, CD8 and the TCR-CD3 complex: a novel class of protein-tyrosine kinase receptor. Immunol Today 11: 400–406

Sadick M D, Heinzel F P, Shigekane V M, Fisher W L, Locksley R M (1987) Cellular and humoral immunity to Leishmania major in genetically susceptible mice after in vivo depletion of L3T4 + T cells. J Immunol 139: 1303–1309

Schubert U, Strebel K (1994) Differential activities of the human immunodeficiency virus type 1-encoded Vpu protein are regulated by phosphorylation and occur in different cellular compartments. J Virol 68: 2260–2271

Swain S L (1981) Significance of Lyt phenotypes: Lyt2 antibodies block activities of T cells that recognize class 1 major histocompatibility complex antigens regardless of their function. Proc Nat Acad Sci USA 78: 7101–7105

Tremblay M Meloche S, Gratton S, Wainberg M A, Sekaly R P (1994) Association of $p56^{lck}$ with the cytoplasmic domain of CD4 modulates HIV-1 expression. EMBO J 13: 774–783

Wofsy D, Carteron N L (1990) CD4 antibody therapy in systemic lupus erythematosus. Sem Immunol 2: 419–425

List of Contributors

(Their addresses can be found at the beginning of their respective chapters.)

List of Contents