CRC

MIGRATION *and* HOMING *of* LYMPHOID CELLS

Volume II

Alan J. Husband

CRC PRESS

Migration and Homing of Lymphoid Cells

Volume II

Editor

Alan J. Husband, Ph.D.
Associate Professor of Immunology
Faculty of Medicine
University of Newcastle
Newcastle, Australia

CRC Press, Inc.
Boca Raton, Florida

Library of Congress Cataloging-in-Publication Data

Migration and homing of lymphoid cells.

Includes bibliographies and index.
1. Lymphocytes. 2. Cell migration. I. Husband, Alan, J., 1949- . [DNLM: 1. Cell Movement. 2. Immunity, Cellular. 3. Lymphocytes—physiology. QW 568 M636]
QR185.8.L9M54 1988 612'.112 87-15125
ISBN 0-8493-6506-6 (set)
ISBN 0-8493-6508-2 (v. 1)
ISBN 0-8493-6509-0 (v. 2)

Direct all inquiries to CRC Press, Inc., 2000 Corporate Blvd., N.W., Boca Raton, Florida, 33431.

International Standard Book Number 0-8493-6506-6 (set)
International Standard Book Number 0-8493-6508-2 (v. 1)
International Standard Book Number 0-8493-6509-0 (v. 2)

Library of Congress Card Number 87-15125
Printed in the United States

PREFACE

Lymphoid cell migration is crucial to successful immune defense. The continued recirculation of small lymphocytes maximizes the opportunity for antigen-presenting cells, effector-cell precursors, and regulatory cells of appropriate specificity to cooperate in response to antigen encounter, and the subsequent migration of effector cells to target sites ensures an appropriate dissemination of the response. Considerable evidence has accumulated in recent years proving that, while this process may be random within specified compartments, there are pools of lymphocytes, and perhaps even of antigen-presenting cells, defined by nonrandom patterns of migration with respect to tissue specificities and antigen-influenced events. An understanding of the restrictions on cell migration is essential to the development of effective immunization strategies.

This book addresses the issues of lymphocyte recirculation leading to inductive interactions in the immune response to antigen, the sites of these interactions, and the subsequent migration and homing of effector cells generated from these responses. In view of the lack of success in establishing effective vaccines against diseases at mucosal sites, particular attention is given to the apparent contrasts between systemic and mucosal lymphoid cell pools, and explanations are sought for mechanisms mediating selectivity of migration and homing.

The contributors to this book represent a wide range of expertise from many research centers ensuring coverage of a diversity of interests on cell-traffic research and providing a broad perspective on this key function of the immune system. Regrettably, a contribution by Professor W. L. Ford was prevented by his untimely death, but his additions to our understanding of cell migration remain an enduring bequest to immunology.

Alan J. Husband, Ph.D.

THE EDITOR

Alan J. Husband, Ph.D., is Associate Professor of Immunology in the Faculty of Medicine of the University of Newcastle in Australia. Dr. Husband received his B.Sc.Agr. degree from the University of Sydney in 1972 and subsequently was awarded a Ph.D. degree from the same university for studies in ruminant immunity. He then spent a period of overseas postdoctoral study in the Sir William Dunn School of Pathology at the University of Oxford and returned to Australia in 1977 to the position of Research Scientist with the New South Wales Department of Agriculture. In 1980 he accepted his appointment at the University of Newcastle.

Dr. Husband's research interests in immunology have focused primarily on problems of immune function at mucosal surfaces, particularly the role of cell migration in mucosal effector responses, and he has published extensively in this area. He is a Member of the Australian Society for Immunology, the Australian Society for Microbiology, and the American Association of Immunologists.

CONTRIBUTORS

John Bienenstock, M.D.
Professor
Department of Pathology
McMaster University Medical Center
Hamilton, Ontario, Canada

Richard M. Binns, Vet.M.B., Ph.D.
Senior Principal Scientific Officer
Department of Immunology
A.F.R.C. Institute of Animal Physiology
and Genetic Research
Babraham, Cambridge, England

Margaret L. Dunkley, M.Sc.
Research Officer
Faculty of Medicine
Royal Newcastle Hospital
University of Newcastle
Newcastle, Australia

Peter B. Ernst, D.V.M., Ph.D.
Assistant Professor
Department of Pathology
McMaster University Medical Center
Hamilton, Ontario, Canada

Delphine Guy-Grand, M.D.
INSERM
Hopital des Enfants-Malades
Paris, France

Alan J. Husband, Ph.D.
Associate Professor
Faculty of Medicine
Royal Newcastle Hospital
University of Newcastle
Newcastle, Australia

Michael E. Lamm, M.D.
Professor and Chairman
Institute of Pathology
Case Western Reserve University
Cleveland, Ohio

Reinhard Pabst, M.D., Ph.D.
Professor
Centre of Anatomy
Medizinische Hochschule Hannover
Hannover, West Germany

Julia M. Phillips-Quagliata, Ph.D.
Associate Professor
Department of Pathology
New York University School of Medicine
New York, New York

John D. Reynolds, Ph.D.
Associate Professor
Department of Medical Physiology
University of Calgary
Calgary, Alberta, Canada

Raffaele Scicchitano, M.D., Ph.D.
Postdoctoral Research Fellow
Department of Pathology
McMaster University
Hamilton, Ontario, Canada

Andrezej Stanisz, M.D., Ph.D.
Assistant Professor
Department of Pathology
McMaster University
Hamilton, Ontario, Canada

Jeenan Tseng, Ph.D.
Chief
Department of Experimental Pathology
Walter Reed Army Institute of Research
Washington, D.C.

Pierre Vassalli, M.D.
Professor and Chairman
Department of Pathology
University of Geneva
Geneva, Switzerland

TABLE OF CONTENTS

VOLUME I

TABLE OF CONTENTS

VOLUME II

Chapter 10

A COMMON MUCOSAL IMMUNE SYSTEM REVISITED

Raffaele Scicchitano, Andrzej Stanisz, Peter Ernst, and John Bienenstock

TABLE OF CONTENTS

I. INTRODUCTION

Mammalian mucosal surfaces are continuously exposed to the environment and have developed a variety of protective mechanisms, both immunological and nonimmunological, to guard against pathogens and damage resulting from absorption of foreign antigens. The basis of specific protection at mucosal surfaces is "secretory immunity", a term which implies differences to the immune responses which arise following systemic exposure to antigen. Early studies showed that the immune response following antigen exposure at mucosal surfaces differs and, to some extent, is divorced from the systemic response. Besredka[1] showed that, following immunization with *Shigella* organisms in both man and rabbits, local humoral immunity exists in the gut independent of systemic antibody responses. Burrows and Havens[2,3] demonstrated in guinea pigs and humans that resistance to *Vibrio cholerae* was related to antibodies (termed copro-antibodies) found in secretions. More than 50 years ago, studies by Bull and McKee[4] showed that if antigen is introduced into the respiratory tract, a local immune response unaccompanied by serum antibody could be formed. Fazekas de St. Groth and Donnelley[5] showed that resistance to influenza virus in mice is correlated to local secretory antibodies and, most significantly, that local antigens are more effective at eliciting these antibodies than parenteral immunization. Thus, the production of local protective antibodies may be independent of the serum antibody response and, when both a systemic and mucosal response are seen, immunity may be best correlated with the local secretory response.

The concept of a distinct secretory immune system was reinforced by the finding that the predominant antibody in secretions differs from the immunoglobulins which characterize serum. Heremans et al.[6] first characterized a new type of immunoglobulin, β_{2A}-globulin (now designated IgA) in serum, and Hanson[7] showed that it predominated in milk and had unique characteristics. Tomasi and Zigelbaum[8] described IgA as the main immunoglobulin in external secretions, and Tomasi et al.[9] showed that it possessed an additional antigenic component — secretory piece [now designated secretory component (SC)]. A further component of IgA in secretions — J chain — was later identified.[10] Both IgA and J chain are produced by plasma cells, while SC is synthesized by epithelial cells.[11] Of particular importance was the finding that, at least in the intestinal and respiratory tracts, IgA is produced in local plasma cells found in the lamina propria of these surfaces. IgA may also be found in secretions of mucosal surfaces where few IgA plasma cells are detected.

The finding that secretory IgA (sIgA) is the predominant immunoglobulin in external secretions led to the concept of an immune system characteristic of external secretions.[9]

Another hallmark of mucosal surfaces is the presence of organized collections of lymphoid tissue with functional and structural characteristics which differ from systemic lymphoid tissue such as lymph nodes and the spleen. In the gut, this mucosa-associated lymphoid tissue (MALT) is represented by Peyer's patches (PP) and the so-called solitary lymphoid nodules. With the rediscovery of lymphoid aggregates resembling PP in the lung [termed bronchus-associated lymphoid tissue (BALT)], it was suggested that the concept of a secretory immune system characteristic of external secretions be extended to that of a universal immune system linking all mucosal surfaces.[12-14] The concept of the common mucosal system arose with the demonstration that PP and BALT are sources of precursors for IgA plasma cells in the gut and respiratory tract,[14] and that exposure to antigen at one mucosal surface leads to dissemination of the response to other mucosal surfaces. Initially, most of the emphasis was on the IgA response and the relocation of IgA plasma cell precursors to distant mucosal surfaces. Recent data showing that circulating dimeric IgA may be transported into external secretions by selective mechanisms have led to an extension of this concept.[15-18] The aim of this review is to critically evaluate the evidence for a common mucosal immunologic system.

This paper deals respectively with the humoral and cellular aspects of a common mucosal immunologic system. The evidence that specific antibodies may be found in secretions following antigenic stimulation at a remote mucosal site is discussed with particular emphasis on the source of these antibodies vis-à-vis synthesis in local plasma cells (themselves possibly derived from remote sites) or derivation from the circulation. We then discuss the cellular aspects and evidence for a cellular common mucosal immune system. It is the opinion of the authors that the existence of MALT and an intermucosal traffic of immunocompetent cells is *prima facie* evidence for, and central to, the concept of a common mucosal immune system.

II. HUMORAL ASPECTS OF A COMMON MUCOSAL IMMUNOLOGIC SYSTEM

A. Immunoglobulin A

IgA is the predominant antibody found in most external secretions, although there are species and organ differences.[19-22] For example, in ruminants, the predominant immunoglobulin in mammary secretions, particularly during the phase of colostrum formation, is IgG_1.[23] A major source of the IgA found in these external secretions is the abundant population of IgA plasma cells in the lamina propria of mucosal and glandular tissues.[19,24-26] Plasma cells in mucosal tissues produce IgA as a dimer incorporating J chain ($[IgA]_2$);[11] this $[IgA]_2$ complexes with SC synthesized by epithelial cells, and the SC-$[IgA]_2$ is transported into secretions.

1. Hepatobiliary Transport of IgA

It has generally been assumed that the IgA found in external secretions is produced locally by plasma cells at the mucosal surfaces. This assumption has been based on the presence of abundant IgA plasma cells in mucosal tissue, the fact that serum IgA levels are lower than those in external secretions, and the demonstration of IgA synthesis by mucosal tissues in vitro.

The finding that bile contains large amounts of sIgA derived from the circulation, largely based on experiments in rats, and some other species, suggests that the view that the presence of sIgA invariably indicates local synthesis must be reconsidered.[27,28]

It is now well documented that, in rats, circulating oligomeric IgA[15,16] but not monomeric of sIgA[29-31] is selectively transported into bile by a SC-mediated mechanism (Figure 1).[32-35]

There is little doubt that this hepatobiliary transport of IgA plays a significant biological role, particularly in rats, and it has been estimated that 90% of sIgA in intestinal secretions in this species is derived from bile.[36] Initial studies employed plasmacytoma-derived IgA, but it has now been demonstrated that polyclonal IgA with defined antibody activity[30,37,38] and $[IgA]_2$-antigen complexes[29,39] are also transported selectively into bile. Few studies have examined the transport of $[IgA]_2$-antigen complexes into secretions other than bile. Russell et al.[39] found no evidence for their transport into milk, bronchial, or intestinal secretions in mice. Bienenstock and Befus[40] have suggested that the primary role of oligomeric IgA is to clear from the circulation soluble and small particulate materials that have crossed the epithelia of various mucosal surfaces. If a similar selective transport of circulating $[IgA]_2$ occurs at other mucosal surfaces, it suggests an explanation for how glandular mucosal tissues, not normally exposed to antigen, may receive both specific antibody and antigenic stimulation from distant mucosal sites. It is still uncertain to what extent circulating $[IgA]_2$ is transported selectively into secretions other than bile, and the evidence is reviewed below.

The biological significance of this "hepatobiliary pump" in species other than rats is uncertain. For example, in man, SC and IgA have been detected in bile duct epithelium,

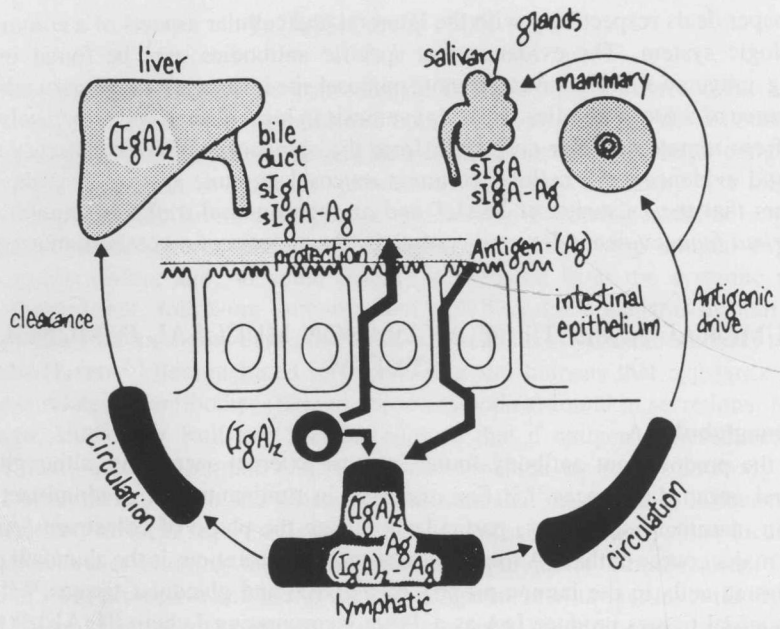

FIGURE 1. This diagram integrates work performed in animals. Dimeric IgA ($[IgA]_2$) is synthesized by lymphoid cells in the intestinal lamina propria. It is transported into the lumen as secretory IgA (sIgA) after specific complexing with secretory component (SC) synthesized by these cells. Some IgA ($[IgA_2]$) diffuses into the lymphatics and the circulation, from which it is cleared as sIgA by hepatocytes, into the bile (by far the major portion), or by glandular epithelial systems including the intestine, lacrimal, salivary, and mammary glands into their secretions. Other tissues capable of SC synthesis also possess this selective transport ability for the $[IgA]_2$ molecule. Dimeric IgA complexed to antigen, which has penetrated the mucosal barrier, has the same distribution and a similar fate. These complexes may selectively localize in glandular tissue in small amounts and act as an antigenic drive for local IgA-producing lymphocytes. (Reprinted with permission from "Some thoughts on the biologic role of immunoglobulin A", by J. Bienenstock and A. D. Befus, *Gastroenterology*, 84, 178. Copyrighted by the American Gastroenterological Association, 1983.)

but claims for its presence in hepatocytes are controversial.[11] Both monomeric and sIgA are found in human bile, and bile duct obstruction leads to elevated levels of circulating IgA.[28,41] Therefore, it is likely that biliary transport of IgA in man and other species may still prove to be biologically significant.

2. Origin of IgA in External Secretions

Few studies have looked carefully at the question of the local synthesis vs. serum derivation of IgA in external secretions. Several approaches have been used including (1) the comparison of immunoglobulin concentrations in serum and secretions or efferent lymph, and (2) kinetic studies using radiolabeled immunoglobulins.

Using these techniques, it was demonstrated that the majority of IgA in external secretions including the respiratory tract,[42,43] intestine,[44-46] and saliva[22] are derived by local synthesis. Data regarding the selective transport of circulating IgA into these external secretions are more controversial. Scicchitano et al.,[47] in experiments involving the intravenous injection of radiolabeled dimeric IgA in sheep, reported that although the majority of IgA in upper respiratory secretions was derived from local synthesis, a significant amount (approximately 20%) was derived from the circulation by a selective transport mechanism. However, in mice there was no evidence for significant transfer of circulating homologous IgA into

Table 1
CATEGORIES OF TISSUES WHICH CONTAIN SECRETORY IgA OR ITS COMPONENT PARTS

Glands	Tissues
Class I. Evidence of local predominance of IgA plasma cells and secretory component	
Salivary	Gastrointestinal tract
Mammary	Upper respiratory tract
Lacrimal	Nose
Prostate	Middle ear
	Gallbladder
	Cervix
	Biliary duct
	Uterine mucosa
Class II. Evidence for local secretory component, but no evidence for local IgA plasma cells	
Skin (sweat glands)	Amnion
Kidney	Fallopian tubes
Urinary bladder	Thymus
Hepatic parenchymal cells	
Class III. No evidence for either local secretory component or local IgA plasma cells	
Ureter	
Esophagus	
Vagina	
Buccal squamous epithelium	
Gingiva	

From Bienenstock, J. and Befus, A. D., *Gastrointestinal Immunity for the Clinician*, Shorter, R. G. and Kirsner, J. B., Eds., Grune & Stratton, Orlando, Fla., 1985, 1. With permission.

bronchial secretions obtained by bronchial lavage. There is also evidence that circulating IgA is selectively transferred into saliva.[18]

With respect to the mammary gland, conclusions from data obtained from these types of experiments are more difficult to derive and may be more open to interpretation. In sheep and mice, in early lactation, the majority of IgA in milk appears to be derived from the circulation by a selective transport mechanism, and these data are consistent with the paucity of IgA plasma cells found in the gland at this time.[17,48-50] When the gland is directly antigenically stimulated, or during involution, increased numbers of IgA plasma cells are present and the bulk of IgA in mammary secretions is from local synthesis.[17,23,26] In contrast to the above findings of Halsey et al.,[49] Russell and co-workers[39] were unable to demonstrate significant transport of circulating IgA into milk (or indeed any other external secretions other than bile) in mice. In rats, specific antibodies to *Escherichia coli* were detected in bile but not milk after their intravenous injection.

Bienenstock and Befus[51] have classified mucosal tissues into three categories (Table 1): class 1 consists of tissues in which there is SC and a predominance of IgA-containing plasma cells; class 2 consists of those sites where SC is found but where there is no predominance

of IgA plasma cells; and class 3 consists of tissues which do not contain SC or IgA plasma cells but which are bathed by secretions containing sIgA. The selective transport of IgA from the interstitial space into secretions probably takes place in all tissues in classes 1 and 2. However, the proportion of IgA in secretions which is serum-derived will reflect the concentration and molecular form of circulating IgA, the availability of SC, and the degree of competition from IgA produced locally. Thus, if the majority of the serum IgA was derived from the intestine, it would be expected that most of it would be dimeric and hence in the appropriate molecular form to bind SC and be translocated to the lumen. This is indeed true in many species including dogs,[44] rodents,[52] and sheep.[45]

In this situation, a large proportion of IgA in exocrine secretions may be derived from the circulation if there are few plasma cells found locally. Therefore, in the intestine where there are abundant IgA plasma cells, little of the IgA in secretions derives from the circulation. In contrast, in the mammary gland, there are relatively few IgA plasma cells present in early lactation and the majority of the IgA in milk comes from the circulation. The reverse situation occurs during involution when abundant IgA-synthesizing plasma cells are found locally.

3. Significance of Selective Transport of IgA into External Secretions

Much of the evidence for the common mucosal immunologic system reviewed below concerns the finding of specific antibodies, usually following oral immunization, in the external secretions of remote mucosal sites in the *absence of serum antibody*. This latter fact has been taken to suggest that this antibody had originated at distant mucosal surfaces presumably by relocation of primed IgA plasma cell precursors. It is clear now that these assumptions are not necessarily valid. While they may be true, two alternative hypotheses must be entertained: (1) mucosal antibody is derived from the circulation by selective or other transport, and (2) antibody-synthesizing cells have migrated to that local site and are there secreting specific antibody. Furthermore, these cells, including IgA memory B cells, may be stimulated not only by local antigenic stimulation, but also by antigen derived from distal mucosal sites in the form of $[IgA]_2$-antigen complexes arriving in the circulation.[40]

All that is required to maintain a low steady-state serum concentration is that the rate of removal of IgA from the serum be faster than its synthesis and secretion. Convincing evidence for this has been shown in rats where SC-mediated transport of $[IgA]_2$ into bile is responsible for the low levels of serum IgA.[53] Recently, Sheldrake et al.,[54] have provided evidence that, in sheep, specific IgA antibodies, probably derived from the intestine, may be found in milk during involution. Most significantly, these antibodies were detected in the absence of specific antibody in the serum and at this time immunohistochemical studies also showed the absence of specific IgA antibody-containing cells (ACC) in the mammary gland, providing conclusive evidence that the antibodies in milk did not come from local synthesis. In addition, specific IgA antibodies in milk from glands which had been immunized locally and control glands which had received no such immunization were comparable.

The selective transport of circulating $[IgA]_2$ into secretions provides a complementary mechanism to relocation of ACC whereby IgA derived at one mucosal site may be found at distant mucosal sites. The fact that antigen may also find its way to remote mucosal tissue by this mechanism means that data regarding local responses need to be interpreted carefully.

B. Humoral Evidence for a Common Mucosal Immunologic System Involving IgA

A number of studies have demonstrated the presence of specific antibody in secretions remote from the initial site of antigenic stimulation. This is often taken to represent evidence for a common mucosal immunity, and the mechanism generally proposed involves the relocation of antibody-synthesizing cells from gut-associated lymphoid tissue (GALT) or BALT to distal mucosal sites. We have already presented evidence that circulating IgA may be selectively transferred into external secretions and that the absence of a serum antibody

response may be due to the rapid removal of circulating IgA primarily into bile. Another possible mechanism may be direct antigenic stimulation by antigen which has gained access to the circulation. The absence of a serum antibody response does not signify that antigen has not gained access to the circulation, as even after intravenous immunization there may not be a specific IgA response.

It is now well documented that the intestinal epithelium in the normal adult is not an impermeable barrier to even quite large molecules.[55] Therefore, after oral immunization, antigen may be absorbed and may directly stimulate the lymphoid tissue at distant mucosal sites. In various experimental situations in which radiolabeled proteins have been injected intravenously, intact antigen has been detected in milk, bile, and, to a lesser extent, saliva.[39,56] Not only are BALT and GALT, by virtue of their specialized epithelium, areas of preferential antigen uptake, but this transport is bidirectional.[57,58] At these sites, circulating antigen may also be excreted into the lumen, a situation in which stimulation of lymphoid tissue must result.

It is well known that breast-fed human infants can experience anaphylaxis from food antigens the mother is consuming.[59] Various noniodine-labeled proteins have been detected in milk in experimental situations, and viruses also have been shown to be secreted in saliva and milk.[60,61] In addition, $[IgA]_2$-antigen complexes could be transported selectively across epithelia which express SC and add local antigen to distal sites (Figure 1).

Circulating macrophages could also have mucosal selectivity. They are known to be able to migrate from the work of Pugh et al.,[62] who showed that they can be found in the thoracic duct after mesenteric lymph node (MLN) removal.

A number of studies have shown that antibodies may be detected in saliva, mammary and respiratory secretions, or tears after exposure of GALT or BALT to antigen either during naturally occurring infection or following immunization.

Evidence for a link between intestinal exposure and a secretory IgA response in colostrum or milk is provided by finding specific antibodies to intestinal commensals or pathogens in these secretions. In patients with *Salmonella typhimurium* infection, IgA antibodies were detected in colostrum in higher titers than those found in serum.[63] A relationship between intestinal antigenic exposure and milk antibodies has been described in a number of other situations. Human milk contains high levels of antibodies against many enterobacterial antigens.[64,65] In studies comparing milk antibodies in Swedish and Pakistani mothers, antibodies to *E. coli* antigens were found with equal frequency in both groups; the predominant antibody class was sIgA, although some IgG and IgM antibodies were also found.[55] However, the Pakistani mothers had more frequent and higher levels of secretory antibodies to enterotoxins of pathogenic *E. coli* and in a few cases to the enterotoxin of *V. cholerae*. Since these enterotoxins are not absorbed systemically, this provides some indirect evidence against direct stimulation of the mammary gland.[65] Ahlstedt et al.[55] suggested that antibodies in milk may reflect the antigenic load of the intestine, a suggestion similar to that made by Clancy and co-workers[66] regarding salivary antibodies and the respiratory tract. Additional support for the existence of an enteromammary axis in man has been provided by the finding that a large proportion of milk lymphoid cells produces specific IgA antibodies against *E. coli* O antigens.[55]

The first direct experimental evidence for the existence of an enteromammary axis was provided by Saif and co-workers.[67,68] In swine infected with the intestinal pathogen, transmissible gastroenteritis virus (TGE), specific IgA antibodies were detected in colostrum or milk in higher titers than in serum. Natural or experimental infection resulted in effective passive protection for the neonate. In contrast, when swine were immunized parenterally with a live attenuated virus, specific antibody activity was primarily or solely associated with IgG and there was less effective passive protection for the neonate. In this situation, antibody titers were highest in colostrum and lowest in milk, levels in serum being inter-

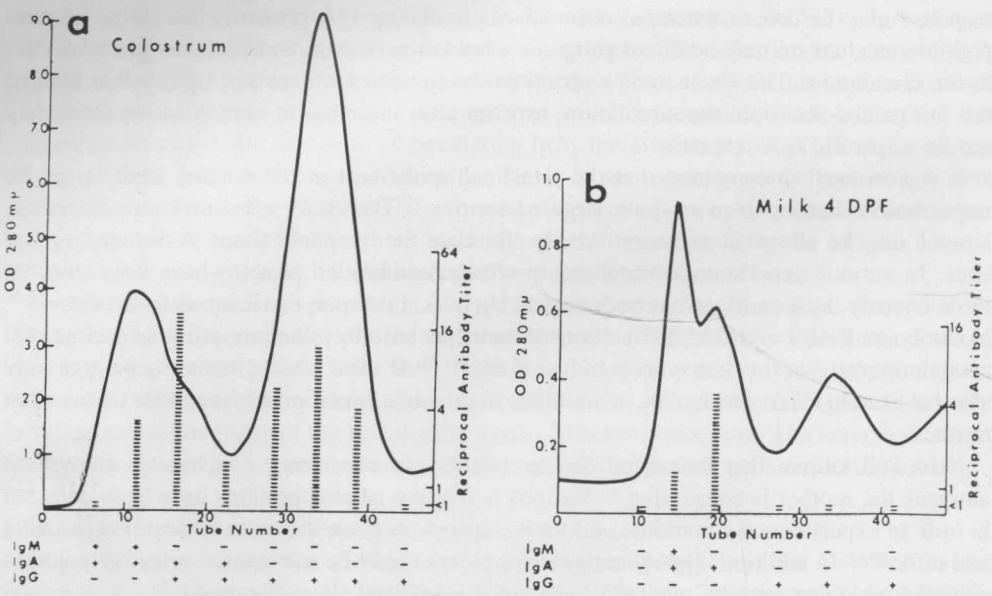

FIGURE 2. Gel filtration on Sephadex G-200 of (a) colostrum and (b) 4-day milk from a sow that had been infected naturally with TGE virus. Indicated are TGE antibody titers, represented by vertical bars, and classes of immunoglobulins in selected unconcentrated eluate fractions. (From Bohl, E. H., Gupta, R. K. P., Olquin, M. V. F., and Saif, L. H., *Infect. Immun.*, 6, 289, 1972. With permission.)

mediate. These experiments suggested that intestinal exposure to the virus was the most effective means of eliciting milk IgA antibodies which provided superior passive immunity. Data from their studies are reproduced in Figure 2.

Experimental proof of a link between the intestine and breast in humans was provided by Goldblum et al.[69] who demonstrated that intestinal colonization of women with a strain of nonpathogenic *E. coli* during late pregnancy resulted in an anti-*E. coli* plaque-forming cell response (primarily IgA) in colostrum within a few days of ingestion of the bacteria. There have been serious reservations expressed about this: Mestecky and co-workers[70] have shown that most of the plaques were from IgA contained within macrophages. However, it has been shown that the plaques are inhibited by protein synthesis inhibitors.[71]

In studies in which pregnant rabbits were immunized by various routes with the hapten dinitrophenyl (DNP) conjugated to bovine gamma globulin and pneumococcus vaccine, in some early work, Montgomery et al.[72] confirmed the link between the intestine and mammary gland. They subsequently extended the concept of common mucosal immunity to include BALT.[73] Both endobronchial and intragastric immunization of pregnant rabbits with these antigens led to an sIgA anti-DNP response in milk, saliva, and intestinal and bronchial secretions, usually in the absence of a serum response. Although the efficiency of stimulation differed between the various routes, intragastric immunization did lead to a specific antibody response in bronchial secretions and in this case specific sIgA antibody represented a significant proportion of the total protein recovered by bronchial lavage. Remote-site stimulation was also achieved in nonpregnant animals.

In a further extension of this work, Montgomery et al.[74] showed that the ocular immune system may be included as part of the common mucosal immunologic system. They studied the specific antibody response in rats immunized by several routes with DNP-pneumococcus vaccine. Intragastric, parenteral, or topical (local) ocular immunization led to IgA antibodies in tears, saliva, and intestinal and bronchial secretions. Using a highly sensitive radioimmunoassay, no specific IgA antibodies were detected in serum regardless of the route of

immunization. In some elegant studies, they determined the clonotypic spectra of the IgA antibodies in secretions by means of isoelectric focusing. Following intragastric immunization, the antibody spectrotypes in saliva, tears, and bronchial secretions were identical, a fact they interpreted as evidence that IgA plasma cell precursors from GALT had seeded the distal mucosal tissues. The specific antibody spectrotypes in saliva, tears, and bronchial secretions after local ocular immunization showed partial homology, suggesting seeding of distal mucosal tissue with IgA plasma cell precursors as well as antigen dissemination. The origin of the IgA-synthesizing cell precursors could well have been GALT as some antigen would have found its way into the oropharynx. Similarly, it is not known if circulating $[IgA]_2$ or $[IgA]_2$-antigen complexes are transported into rat tears, although IgA-ACC were detected in lacrimal glands after intragastric immunization, suggesting that some of the IgA had come from local synthesis.

Regarding the respiratory tract as a source of remote-site antibodies, in naturally acquired infections with the pathogen respiratory syncitial virus (RSV), antibodies which are predominantly sIgA are found in colostrum and milk in humans.

Following natural reinfection with this virus, there was a rise in antibodies of all classes in the serum and nasopharyngeal secretions, but in milk only sIgA antibody increased.[75] In parallel studies, pregnant rabbits were immunized with RSV or bovine serum albumin by the oral, intratracheal (IT), or intravenous (i.v.) routes.[76] Significant sIgA antibody titers were noted in milk only after oral or IT immunization and in the case of IT immunization, IgG antibody was also detected in intestinal secretions. It is pertinent to the mechanisms involved in this remote-site stimulation that RSV antigen could not be detected in the mammary tissue by immunofluorescence, and IgA-producing cell numbers in the mammary gland were elevated. The absence of demonstrable local antigen and of a serum IgA response in this situation argues for the local synthesis of sIgA antibody present in colostrum or milk.

In similar studies to those outlined above, evidence has been provided for the involvement of other mucosal surfaces in the common mucosal immunologic system. Specific antibodies, predominantly sIgA, have been detected in saliva (and tears) after oral ingestion of *Streptococcus mutans,* an organism implicated in the development of dental caries.[77,78] There is evidence that caries incidence is reduced. Clancy et al.[66] showed that feeding of a polyvalent bacterial vaccine to healthy human volunteers results in sIgA antibodies in saliva. In other studies, healthy human volunteers fed *E. coli* developed IgA antibodies to the O antigen in urine, and in rats when *E. coli* were introduced into the bladder, antibodies could be detected in both serum and bronchial lavage, and there was evidence for the local synthesis of the specific IgA found in respiratory secretions.[79]

Therefore, a number of studies have suggested that stimulation of GALT or BALT can lead to specific antibodies at remote sites. The mechanisms involved in this remote-site stimulation are uncertain. There certainly is evidence to be reviewed below that cells which originate in GALT or BALT and which in the main are destined to differentiate into IgA plasma cells are able to lodge in distal mucosal sites. Another mechanism is likely to be the selective transport of IgA, originating from mucosal tissue, into external secretions.

The question of whether antigen may be systemically absorbed and stimulate a local response is unresolved. The failure of a systemic response suggests that this does not occur. Certainly, local stimulation by any antigen which finds its way to the mucosal site may result in an sIgA response even in the mammary gland which is relatively lacking in lymphoid tissue.[26,67,68]

III. CELLULAR ASPECTS OF A COMMON MUCOSAL IMMUNOLOGIC SYSTEM

In the following sections, cellular evidence in support of a common mucosal immunologic