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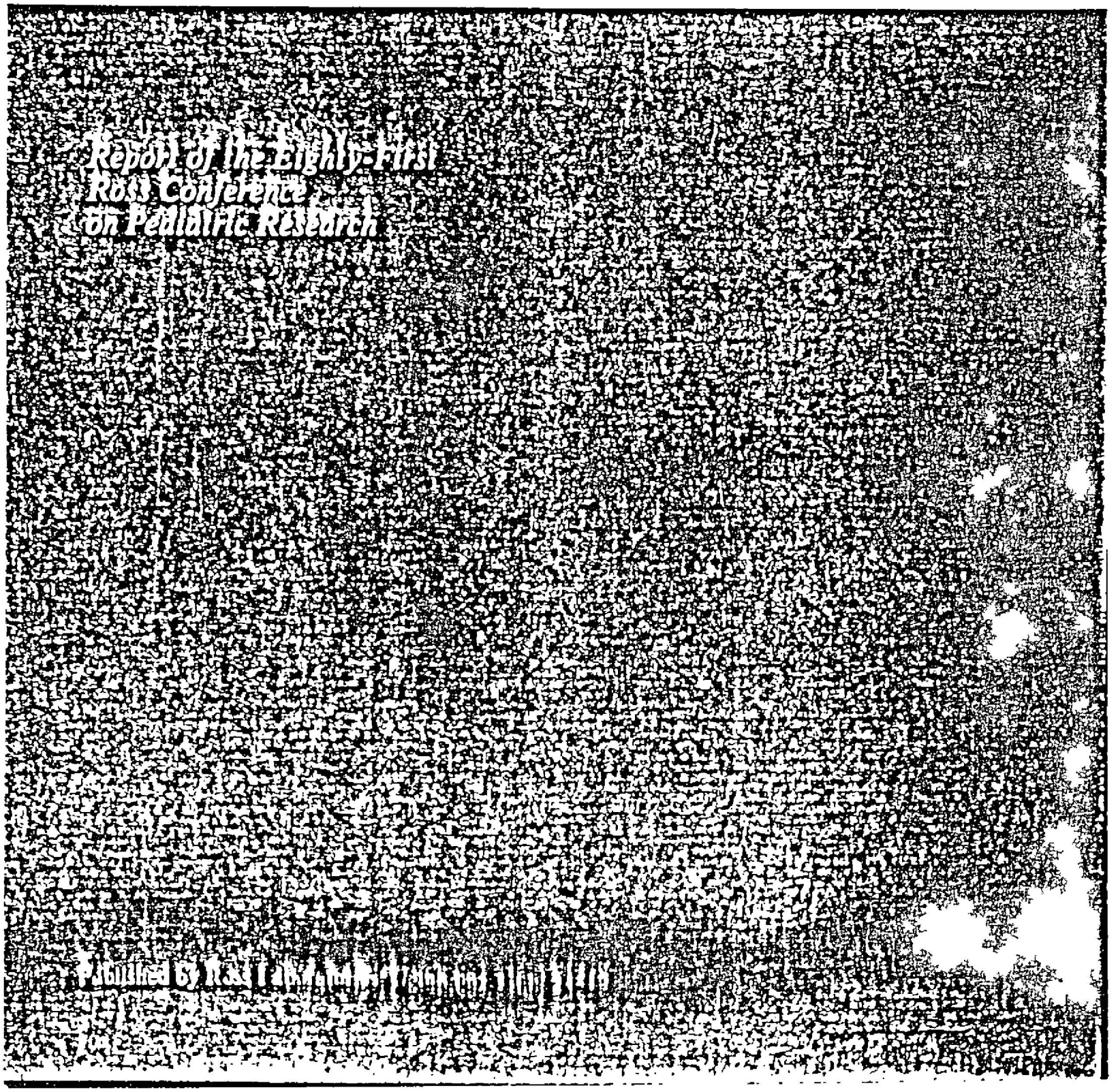
The Mucosal Immune System in Health and Disease



The Enteromammaric Axis of the Secretory IgA System

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Infections, which are a major cause of morbidity and mortality of young infants and children in developing countries, occur primarily after weaning. Epidemics with enteropathogenic *Escherichia coli* in infants can be controlled by human milk. The infant is provided with 0.25 to 0.5 g/day of secretory IgA (sIgA) during breast-feeding. It is likely that milk sIgA functions by preventing microorganisms from attaching to mucosal membranes, since such attachment seems to be a prerequisite for many microorganisms to be able to manifest infection.

Antibodies Against Intestinal Microorganisms in Human Milk

To prevent infections, the maternal milk should contain sIgA antibodies against intestinal pathogens. With few exceptions, milk carries sIgA antibodies against the *E coli* K1 antigen, which is an important virulence factor in 80% of strains causing neonatal meningitis, and sIgA antibodies against certain common pili of *E coli*, important for their mucosal attachment. In samples from Pakistan we have found sIgA antibodies against the enterotoxins of *E coli* and *Vibrio cholerae*, as well as the O antigens of several *Salmonella* and *Shigella*, using the ELISA technique. Intestinal exposure of the mother results in sIgA antibodies appearing in the milk. Intestinal colonization with an *E coli* 083 resulted a few days later in the appearance in the milk of IgA antibodies specific for the colonizing strain. Infection with *Salmonella typhimurium* during pregnancy also induced antibodies detectable in the milk. Experiments in pigs, rats, and rabbits have also shown that intestinal exposure gives rise to milk IgA antibodies.

Milk sIgA and Undernutrition

The antigenic specificity of sIgA milk antibodies from Pakistan, Ethiopia, Guatemala, and Sweden can be used to contribute to epidemiologic information on microbial species, such as polio and rotavirus, and parasites like *Entamoeba histolytica*. Many of the mothers in areas where epidemiologic information is of special interest are undernourished. Severely undernourished Pakistani women had milk sIgA anti-*E coli* comparable to those of healthy Swedish women, but with diminished milk volumes. Their 24-hour output of milk sIgA was therefore decreased. In Guatemala (part of a World Health Organization study of breast-feeding), we found there were no significant differences in the milk sIgA output/24 hours comparing privileged and underprivileged mothers. There was a trend, however, toward lower sIgA concentrations among the undernourished women. The underprivileged mothers had lower sIgA anti-*E coli* levels than the privileged, after compensating for differences in 24-hour milk volumes. The underfed rural women showed a significantly low 24-hour output of milk sIgA antibodies to *Shigella sonnei* but not to *Shigella flexneri*, which is more common (see Table).

Table. Secretory IgA Antibodies Against *Shigella* in Human Milk One Month After Delivery.

Population Group	Number	*Shigella* *flexneri*	*Shigella* *sonnei*
Guatemalan			
Rural	10	102	15
Urban poor	10	124	59
Urban privileged	10	118	58
Swedish	10	71	16

Antibodies determined with ELISA and expressed in percent of a reference. Underlining indicates significantly low levels remaining after compensating for differences in 24-hour milk volumes.

The lower IgA antibody levels among the undernourished may be due to an immunodeficiency secondary to lack of nutrients, to their many infections, or to less antigen exposure. Thus the lower levels of *Salmonella* and *Shigella* antibodies among Swedish women (Table) are most probably due to less exposure to these pathogens or cross-reacting pathogens. The rural Guatemalan women may be less exposed to certain microorganisms than the urban privileged women, who often buy processed foods (tortillas) which have time to become heavily contaminated with gram-negative bacteria before consumption.

The privileged Guatemalan mothers who drink milk and eat meat showed much higher milk sIgA levels than the poor mothers to cow's milk proteins. It is also possible that the two groups of poor women responded less well due to their undernutrition (Figure).

The low levels of sIgA antibodies against *E coli* among the undernourished women induced us to repeat the comparisons of the milk antibody responses after identical exposures to a previously unencountered food protein and also to look for possible effects of concurrent infections.

Milk sIgA Antibodies and Vaccination

After parenteral immunization with a cholera vaccine in previously endemically exposed Pakistani mothers, we found an increase of both serum antibodies and milk and saliva sIgA antibodies. This did not occur in previously unexposed Swedish mothers. Thus, an existing response may be boosted, but it is difficult to induce a primary sIgA response by parenteral immunization. Vaccinations with live oral polio vaccination showed that the milk sIgA antibody levels diminished significantly in 6 of 10 Pakistani women previously naturally exposed. If the oral polio vaccine was given together with parenteral cholera vaccine, the milk sIgA polio antibodies practically vanished. These alarming observations require further studies for evaluation.

Mechanisms for Transfer of the IgA Response From Intestine to Mammary Gland

On the basis of Craig and Cebra's work, an explanation for the appearance of milk IgA antibodies after antigenic exposure in the intestine would be a homing of

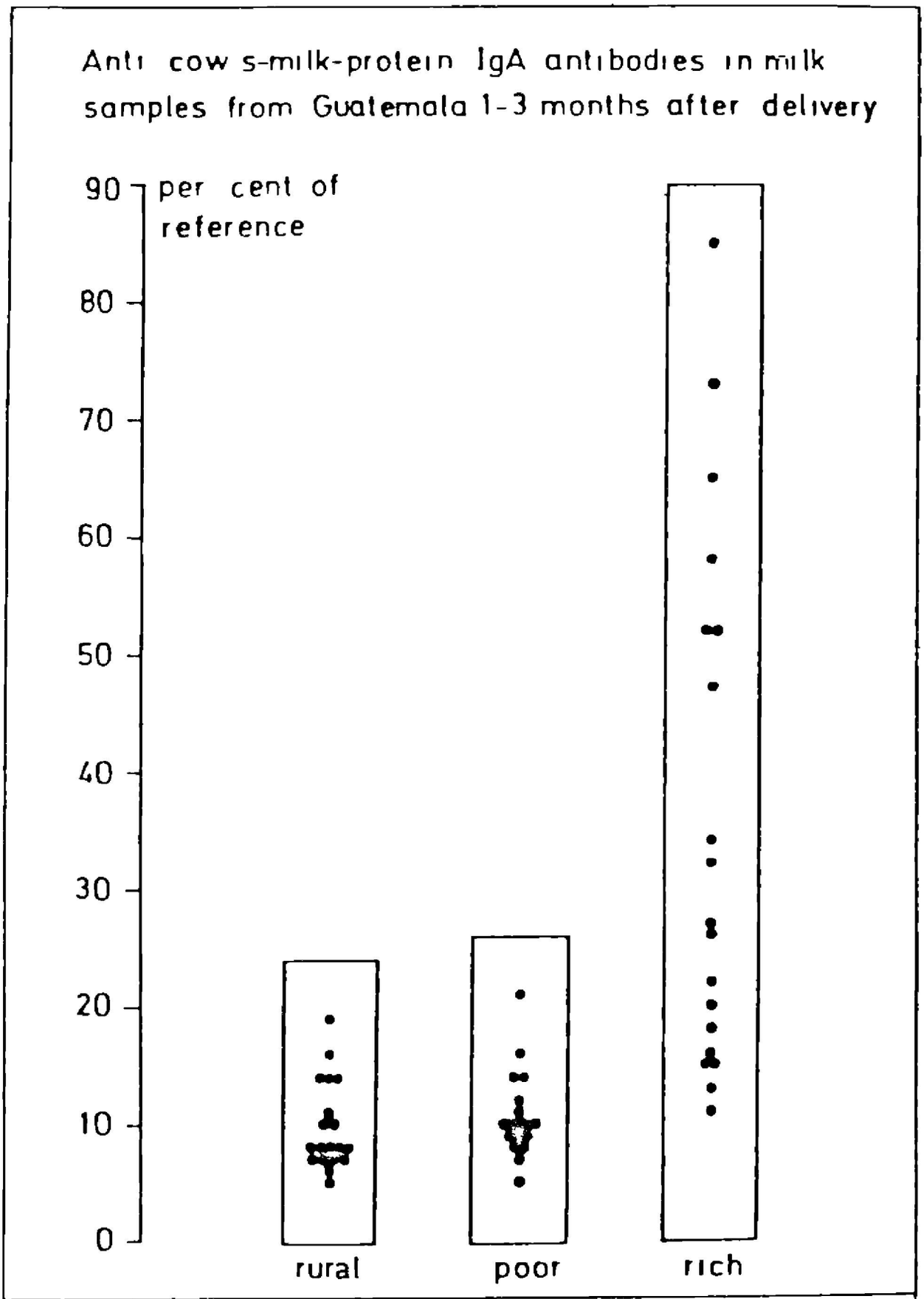

Figure. Measurements of levels of milk IgA antibodies against cow's milk proteins in women of three different socioeconomic groups in Guatemala. The levels were determined with ELISA and expressed in percent of a reference

Peyer s patch cells to the mammary gland The existence of such a homing has been well supported by the studies of Lamm et al and Bienenstock et al The homing cells would be producing IgA antibodies in the gland and correspond to the many lymphoid cells seen in the mammary glands of pigs, mice and rabbits during lactation In the sow, up to 90% of the milk IgA seems to derive from local synthesis

It is possible that the secretory component on the surfaces of the epithelial cells in the lactating mammary gland can contribute by taking up IgA dimers from the blood, possibly with their origin in the intestine.

Recent passive transfer experiments in mice and dogs show such a transfer of IgA into various secretions, including milk. In intestinally immunized rats, we do not see such a transfer of *E coli* IgA antibodies after bile duct occlusion, which results in a significant increase in the serum IgA antibodies. Much of this increase is composed of sIgA antibodies which should not attach to the secretory component molecules functioning as receptors for IgA dimers in the mammary gland. It is possible that the active immunization also leads to an IgA synthesis in the mammary gland which occupies the available secretory component sites required for transfer of IgA dimers from the blood.

In summary, it is apparent that antigenic exposure in the intestine results in sIgA antibodies appearing in the milk. Homing of committed IgA-producing lymphoid cells from the Peyer's patches of the intestine to the gland may be a major explanation, but an uptake of IgA dimers from the circulation may also contribute.

As a result of this enteromammaric axis, breast milk contains sIgA antibodies against all the various food and microbial antigens the mother is exposed to. Consequently, the breast-fed baby is provided with mucosal protection against most antigens and infectious agents to which it is exposed. Furthermore, the milk antibody content determined in population groups can give epidemiologic information about intestinal pathogens. It seems that undernutrition may have only a limited effect on milk sIgA antibody levels, but this requires further study.

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Discussion

DR PIERCE: In the experiment with the polio vaccination, did you determine whether poliovirus was excreted, or was there a failure of the vaccine virus to become established?

DR HANSON: This was just a pilot study, and that was not done. It is obviously important, because there may have been replication of virus in the intestine and antibody consumption or deviation of the cells producing the antibodies away from the mammary gland.

DR ROTHBERG: Do you have any data on the effect of your immunizations on the IgG content of the milk?

DR HANSON: The IgG content in milk in our experiments relates to serum levels by a factor of 0.4, and IgM with a factor of 0.8, whereas the IgA milk-to-serum ratio is more than 10. We do not know the influence of vaccination on milk IgG.

DR PLAUT: I believe it is worth making a point with respect to the hormones which may influence cell traffic. We have been talking about reproductive hormones. If one really wants to look at a hormone factor, one should consider the gastrointestinal tract. There are probably 20 or 30 separate hormone-producing cell types in the GI tract. I just want to point out that some of these hormones whose function is not known in GI physiologic circles may, in fact, be quite important to our understanding of factors affecting lymphocyte localization.

Some of these hormones, locally released, such as somatostatin, may influence other cells in their own environment without entering the circulation.

DR MASSON: I wonder if Ia antigens could play a role in lymphocyte localization, because Ia antigens are expressed on gut epithelial cells. Are Ia antigens expressed in the mammary gland?

DR HANSON: Yes, and they are directed by the same lactogenic hormones that make the epithelium develop. However, it has recently been shown that the Ia antigens may not be very important in cell localization, because in the intestine the Ia antigens are found primarily at the tips of the villi, whereas the IgA-producing cells are mostly at the bases.

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Immunoglobulin-Containing Cells and Noncellular Elements in Human Colostrum

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Ingestion of colostrum or milk provides the neonate with passive humoral and cellular immunity due to the presence of specific antibodies, innate humoral factors (lactoferrin, peroxidase, and lysozyme), phagocytic cells, and lymphocytes.[1] Human colostrum contains approximately 3×10^6 cells/ml, of which 30% to 47% are macrophages, 40% to 60% polymorphonuclear leukocytes (PMN), 5% to 9% lymphocytes, and the rest colostral and epithelial cells.[2] The demonstration,