Vitamin A and retinoids in antiviral responses

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ABSTRACT **Vitamin A deficiency results in multiple derangements that impair the response to infection. This review focuses on experimental models of specific virus infections and on cytokines and cells with cytolytic activity important to antiviral defenses. Altered specific antibody responses and greater epithelial damage in vitamin A-deficient hosts are consistent findings. The cytolytic activity of natural killer cells and various cytokine responses are altered. The inflammatory response to infection may also result in derangements in the transport and metabolism of retinol. We speculate that interaction of several factors may combine to explain the greater severity of infection seen in vitamin A-deficient animals and children. In addition to a preexisting lack of tissue vitamin A, these factors may include reduced mobilization and increased excretion of retinol during the acute phase response to infection, poor innate and specific immune response to virus, and delayed repair of damaged epithelia. Foci of vitamin A-deficient epithelia may be sites of penetration of bacteria and other agents, leading to secondary infections and contributing to an increased severity of infections and poor outcome in vitamin A-deficient animals and humans.—Ross, A. C., Stephensen, C. B. Vitamin A and retinoids in antiviral responses. *FASEB J.* 10, 979–985 (1996)**

Key Words: Newcastle disease virus · Herpes simplex virus · natural killer cells· cytokines · retinol binding protein · vitamin A status

AN ASSOCIATION BETWEEN VITAMIN A and the immune response to infection was postulated in the late 1920s. The impetus for renewed interest in this subject comes from research in several disciplines. First, it is now recognized from clinical and cellular studies of hematopoiesis that retinol, retinoic acid, and related retinoids affect lymphocyte development and maturation (reviewed in ref 1). Second, cell culture and animal nutritional studies have shown that vitamin A and related retinoids are capable of modulating lymphocyte responses that lead to antibody production, T cell activation, and cytokine production (see ref 2 for a general review). Third, the results of numerous epidemiologic studies have shown convincingly that mortality, and sometimes morbidity, are greater in vitamin A-deficient children even when xerophthalmia is not present (3). Diarrhea, measles, and, in some studies, respiratory infections are common in vitamin A-deficient children (4). Diarrhea and respiratory infections are caused by many different etiologic agents, including viruses and bacteria, whereas measles is caused by a single agent, the measles virus. For many vitamin A-deficient children, the cause of death is likely to be an overwhelming infection of viral or bacterial origin. A meta-analysis of eight field-based studies showed a highly significant effect of vitamin A supplementation in preschool children in poor regions of Asia, Malaysia, and Africa, with an overall reduction in mortality of 23% (5). Although most data suggest that vitamin A status has little effect on the incidence of infectious disease, the severity of some infections, especially those associated with diarrhea, may be reduced when vitamin A status is improved (6). In contrast, there seems to be no benefit of vitamin A in reducing the severity of childhood pneumonia in developing countries (7).

In this review, we first consider experimental studies designed to understand the requirements for and functions of vitamin A in the in vivo response to infection with specific viruses. Second, we review the role of vitamin A in maintaining cells and cytokines with antiviral properties, focusing on natural killer (NK)[2] cells and related cytokines. Third, we discuss briefly how infection may alter the normal transport and metabolism of retinol. From these data, we postulate an interplay between vitamin A and infection in which vitamin A deficiency results in derangements of the immune response, while the inflammatory response initiated by infection reduces the mobilization of retinol and its transport to tissues, thereby contributing to tissue depletion of retinoids needed for the repair of infection-damaged epithelia.

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[2]Abbreviations: NK, natural killer; NDV, Newcastle disease virus; IL, interleukin; Th, T helper; HSV, Herpes simplex virus; HIV, human immunotrophic virus; RBP, retinol binding protein.

VITAMIN A DEFICIENCY AND VIRUS-HOST INTERACTIONS

General features

Vitamin A deficiency can affect several aspects of a virus' interaction with its host. Infection begins when a virus binds to its cellular receptor and is internalized. The virus must then "uncoat," replicate its genome, produce structural and nonstructural viral proteins, and assemble into infectious progeny virions that then (typically) exit the cell in order to find another susceptible cell to infect and, eventually, to find another host. The host's initial response to viral infection is through antigen-independent (innate) mechanisms, particularly the production of cytokines of the interferon family at the site of initial infection and the activity of cytotoxic NK cells, which are themselves activated by interferon. After this initial response, the host develops a specific immune response to viral proteins that typically includes both antibody production (leading to viral neutralization) and cell-mediated effector mechanisms, particularly the generation of virus-specific cytotoxic T lymphocytes that kill virus-infected cells. After clearance of the virus, the host must then repair damage to cells and tissues caused by the virus itself as well as the host's inflammatory response to infection. Several aspects of the host response have been examined in experimental animal models, as summarized in **Table 1.**

Newcastle disease virus infection in chickens

The best-characterized model for examining the interaction of vitamin A status and viral infection is Newcastle disease virus (NDV) infection of chickens. NDV is a member of the *Paramyxovirus* genus of the family *Paramyxoviridae*; members of this genus that infect humans include parainfluenza virus types 1 through 4, which cause respiratory infections. Viruses from other genera in this family include the important human pathogens measles virus and respiratory syncytial virus. Like other paramyxoviruses, NDV is transmitted by the respiratory route. Tissue tropism and age of susceptibility of the host vary for NDV strains, but the virus isolates used in the studies described here primarily infect epithelial cells lining the respiratory tract. The age dependence of lentogenic strains (those causing disease in young chicks but not adults) can be altered by vitamin A deficiency in that the high level of virus replication seen in 1-wk-old chicks is maintained through 3 wk of age by feeding chicks a vitamin A-deficient diet (8). Vitamin A metabolites promote the differentiation of epithelial cells in the respiratory tract. These authors speculated that delayed epithelial maturation in vitamin A-deficient animals, rather than a direct effect of vitamin A on the virus or on the immune response, accounted for the increased shedding of virus at 3 wk of age. This age dependence may be due to expression of a trypsin-like enzyme in the respiratory epithelium, which is necessary to cleave the F_0 protein of the NDV virion and render it infectious (9).

The effect of vitamin A deficiency on the replication of mesogenic NDV strains (those that produce disease in infected adult chickens) is not certain. Such NDV strains do produce more severe clinical signs of infection (respiratory signs, general weakness) in vitamin A-deficient animals than they do in control animals (10, 11). Increased virus replication could account for this increased virulence, but other factors, such as a decreased immune response or impaired epithelial recovery, could also be important. Mortality is also increased in adult chickens fed vitamin A-deficient diets, at least when the deficiency is advanced enough to impair weight gain in uninfected animals (10).

The immune response to NDV infection is also impaired by vitamin A deficiency. The total number of both B and T lymphocytes is decreased in the peripheral blood as well as in both primary and secondary lymphoid or-

TABLE 1. *Summary of effects of vitamin A deficiency on host response to viral infections in animal models*

Aspect of virus infection or host response	Principal effect of vitamin A deficiency
Virus replication	Not affected? (little data)
Interferon-α and -β response at site of initial infection	Unknown
NK cell activity	Decreased (decreased number of cells)
Serum IgG and IgM response	Often not affected; possible increase?
Secretory IgA response	Decreased
Cell-mediated immune response	Decreased? (little data)
Regeneration of virus-damaged epithelia	Severely impaired
Severity of infection	Increased severity of clinical signs; probable increase in mortality for some infections

gans (12, 13). Lymphopenia normally occurs during the acute phase of NDV infection, followed by a rebound to greater than normal levels. This pattern—both the decrease and subsequent increase of lymphocytes—is blunted by vitamin A deficiency. That the NDV-specific cytotoxic T lymphocyte response is also impaired by vitamin A deficiency is particularly important. The activity of cytotoxic T lymphocytes against NDV-infected cells is decreased both during the primary infection and during a secondary challenge infection by approximately 40% (14). Such a decrease measured in vitro suggests that virus clearance in vivo may also be affected, although this was not directly demonstrated.

In contrast, the serum antibody response to both a primary and secondary NDV infection is not affected by vitamin A deficiency, although addition of supplemental retinoic acid to the diet can increase the secondary response above control levels (12, 15, 16). Similarly, the serum antibody responses of chickens to another viral respiratory pathogen (infectious bronchitis virus) and to an enteric pathogen (reovirus) are not affected by vitamin A deficiency (17).

The NDV-specific secretory IgA response has not been examined; however, the concentration of total IgA in bile is decreased in vitamin A-deficient animals while levels in the respiratory tract (tracheal homogenates) are not affected (15, 16).

Recovery from NDV infection involves regeneration of respiratory epithelium, which has been damaged by virus replication and the immune response. This process is dramatically impaired by vitamin A deficiency. A hallmark of vitamin A deficiency is the replacement of the normal ciliated cuboidal or columnar epithelium and mucus-secreting goblet cells by a keratinized squamous epithelium (18, 19). Such metaplastic changes may be important in the response to infection because these metaplastic sites in the airways are more susceptible to colonization by some bacterial respiratory pathogens. This colonization could increase the risk of secondary bacterial infection after a primary viral infection, as is often the case after an episode of measles (20) or other respiratory viral infections (21).

Rotavirus infection of mice

Rotavirus is an important human pathogen that causes diarrheal disease in children generally under 2 years of age. Human rotavirus isolates have only recently been adapted to limited growth in mice, but a naturally occurring mouse rotavirus has been used as a model system (22). As with NDV infection of the respiratory tract, the most dramatic effect of vitamin A deficiency on the host's response to rotavirus infection is the inability of deficient animals to repair virus-induced damage to the intestinal epithelium. The cytopathic effects of rotavirus infection are much more severe in the majority of vitamin A-deficient animals than in vitamin A-sufficient controls, with the villous tips in some areas of the small intestine being completely destroyed, leaving the lamina propria exposed to the gut lumen. This level of pathology was not seen in uninfected, deficient animals or in infected animals on control diets (22).

It was also noted that the serum antibody response was decreased in vitamin A-deficient animals; however, the secretory IgA response, which is important in protection against reinfection by enteric pathogens, was not measured (23). Cell-mediated immunity, measured by dermal sensitization of animals with picryl chloride 1 day after rotavirus infection, and the subsequent delayed-type hypersensitivity response to rechallenge with picryl chloride were decreased by approximately 30% (24). Although such delayed-type hypersensitivity responses are not a key component of an antiviral response (but are involved in the clearance of other intracellular pathogens, such as tuberculosis bacilli), they have been traditionally used to measure the effect of malnutrition on cell-mediated immunity and suggest the impairment of cell-mediated immunity during vitamin A-deficiency.

Influenza A virus infection of mice

Influenza A virus causes mild to severe respiratory infections in humans. Some strains of influenza A have been adapted to mice by serial passage. Infection of mice in the upper respiratory tract can cause a mild infection whereas inoculation of the lungs can cause severe viral pneumonia. Virus replication and the rate of virus clearance from the respiratory tract are not affected by vitamin A deficiency in this model (25). Nor does the extent of inflammatory lesions during viral pneumonia differ between deficient and control mice. As with NDV infection, however, regeneration of the normal epithelium is impaired and florid metaplastic lesions are seen in areas where virus replication resulted in inflammation and cellular damage. Adjacent areas are histologically normal. In spite of this, animals recover and there is no difference in mortality or in the clinical severity of infection, at least in mildly vitamin A-deficient animals (i.e., before they have reached a weight plateau as a result of vitamin A deficiency).

The influenza-specific serum IgG response, which helps protect the lungs against infection, is either not affected or may be increased by twofold in vitamin A-deficient animals (26). This increase in mice is largely due to an increase in IgG2a, an isotype that predominates in the response to viral infections. The murine IgG2a response is driven by interferon-γ-producing T helper (Th) cells designated as type 1 (Th1) cells (27). In contrast, the influenza A-specific IgA response, which helps protects the upper respiratory tract against infection, is dramatically diminished in vitamin A-deficient mice, with salivary IgA levels being less than 10% of those in animals fed control diet. The expression in mice of the polymeric total IgA (as compared to influenza A-specific IgA) concentrations in saliva and bile are higher in vitamin A-deficient mice than controls. This is apparently due to in-

creased expression of the polymeric immunoglobulin receptor, which transports IgA across mucosal surfaces and into the bile (C. B. Stephensen, unpublished observations). This increase differs from the reduction in biliary and intestinal IgA levels found in vitamin A-deficient chickens (15, 16) and rats (28–30). The decreased influenza-specific IgA appears to be due to a reduction in the number of antibody-secreting plasma cells (C. B. Stephensen, unpublished observations), as has been reported in other systems for serum antibody response against purified protein antigens (24). The IgA response is driven mainly by T cells of the Th2 type, characterized by a high level of production of interleukin-4 (IL-4) and IL-5 (27). The decreased influenza-specific IgA response is consistent with work from other laboratories indicating that antibody responses driven by Th2 cells are often diminished by vitamin A deficiency in inbred mice, whereas interferon-γ production by Th1 cells from vitamin A-deficient mice may be increased in vitro (31). This increased production of interferon-gg, if it also occurs in vivo, might explain the higher IgG2a response of vitamin A-deficient mice.

Herpes virus infection of rats

Herpes simplex virus (HSV) infects humans and other species, commonly causing ulcerative lesions on mucosal surfaces (herpes labialis and urogenital infections) but also encephalitis and keratitis. HSV causes both acute and latent infections, the latter being reactivated by numerous stimuli. Nauss et al. (32) investigated the effects of vitamin A deficiency on ocular infections in HSV-infected rats. HSV infection of the cornea induced an inflammatory cell infiltrate, first producing corneal opacity, followed by epithelial ulceration and stromal degeneration with eventual perforation; the severity of lesions is determined by the quantity of virus inoculum.

After large inocula, ocular pathology developed more rapidly in vitamin A-deficient animals than in controls, but eventually the extent of damage was similar. With smaller inocula, a higher percentage of vitamin A-deficient animals developed lesions and the severity of these lesions was greater, as judged by slit-lamp scores and the extent of microscopic pathologic changes. The greater pathology in the deficient rats did not appear to be due to a decreased cell-mediated immune response to HSV because the HSV-specific proliferative response of cells in draining lymph nodes and the spleen of deficient animals was equal to or substantially greater than in the control animals at all postinfection time points (33). This more intense immune response could have been triggered by a higher level of virus replication in the deficient rats, but virus replication was not measured in these experiments.

Splenic NK cell cytolytic activity (against heterologous target cells) was decreased by approximately 30% in vitamin A-deficient, HSV-infected animals. Diminished NK activity could have impaired the initial host response to HSV infection, and this may have contributed to the greater severity of infection found in these vitamin A-deficient animals. The serum antibody response to HSV was not affected by vitamin A status. Parenteral administration of vitamin A to nondeficient rabbits decreased the severity of HSV keratitis (34, 35). This beneficial effect of supplemental vitamin A could be due either to improved healing of HSV-induced epithelial lesions or to some enhancement of the immune response.

Retroviral infections

No animal model studies have examined the effect of vitamin A on infection with human immunodeficiency virus (HIV) or its simian counterpart, SIV. However, one study with a murine retrovirus reported increased survival of mice fed diets supplemented with very high levels of vitamin A, and the investigators suggested that enhancement of the immune response was important in improving survival (36). With respect to HIV infection, the long-terminal repeat region of the HIV virus contains a functional retinoic acid response element (37). Retinoic acid treatment in vitro can either increase or decrease HIV replication, depending on the cell types used (38). The implications of such regulation for viral pathogenesis are not yet clear, but these data suggest that the replication of HIV as well as other viruses with DNA genomes or intermediates (39) can be directly modulated via the activity of retinoid receptors.

ANTIVIRAL CELL-MEDIATED CYTOLYSIS AND CYTOKINE PRODUCTION

As indicated earlier, the initial response to viral infections is largely mediated by nonspecific mechanisms, including the cytolytic action of NK cells against virus-infected host cells. NK cells are a quantitatively minor population of lymphocytes found predominantly in blood, spleen, and liver that have abundant cytoplasmic granules that contain the pore-forming protein perforin and a number of proteases. The cytolytic activity of NK cells is increased substantially upon exposure to IL-2 or interferon (mainly α and β). NK cell are capable of a rapid response, without prior sensitization, to virus-infected cells (or heterologous cells used as targets in vitro) through the release of their lytic mediators. Activated NK cells are also major producers of interferon-γ and tumor necrosis factor-α and thus have a potentially major role in regulating other immune responses, including antibody production. In general, but in a manner that may differ for specific infectious agents, interferon-γ (e.g., from NK or Th1 cells) facilitates cell-mediated responses and may inhibit the antibody responses facilitated by cytokines produced by Th2 cells.

Experiments by Nauss and Newberne (33) showed reduced NK cell cytotoxicity in splenic lymphocytes from vitamin A-deficient, HSV-infected rats. In uninfected rats, NK cell activity was also reduced by 30 to >50% in vitamin A-deficient rats (40, 41), and restored to normal levels by dietary treatment with retinol or retinoic acid

(42). However, it was not clear whether reduced NK cell activity resulted from low cytolytic activity per cell or a reduction in the number of the NK cell phenotype. Using a monoclonal antibody that recognizes rat NK cells, Zhao et al. (41) showed that the majority of the reduced NK cell cytotoxic activity in peripheral blood and nearly half of the reduction in spleen could be explained by a low number of NK cells. Nonetheless, NK cells from both vitamin A-deficient and control rats were activated essentially equally by interferon-α/β or IL-2, which suggests that although cell numbers were reduced, possibly due to impaired lymphopoiesis and NK cell maturation, the functional activity of NK cells reaching maturity was not impaired.

There is little information concerning NK cells in children with vitamin A deficiency. Griffin et al. (43) noted reduced NK cell activity in the peripheral blood of Peruvian children with measles, which remained low for at least 3 wk after the onset of rash. The ability of NK cells to respond to IL-2 was retained. Although the vitamin A status of these children was not determined, it is tempting to speculate that it may have been marginal. In vitamin A-deficient rats treated with dietary retinoic acid or N-(4-hydroxyphenyl) retinamide, the number of NK cells returned to normal within a week (42). Retinoids may also stimulate the cytotoxicity of NK cells from normal animals, increasing NK cell lytic efficiency (cytolytic activity per NK cell) (42). A possible mechanism for the reduced cell-mediated responses to viruses and other infectious agents in vitamin A-deficient animals and children may be related to impaired production or maturation of NK cells, reducing their immediate impact via cytolysis and possibly resulting in a reduction in the secretion of cytokines that participate in the regulation of antigen-specific antibody responses.

Evidence from in vivo studies indicates that the production of cytokines may also be altered during vitamin A deficiency. Cells from vitamin A-deficient mice produced less IL-4 and IL-5, consistent with reduced antibody responses. Conversely, IL-12 and interferon-γ were expressed constitutively in deficient mice; these effects were proposed to shape the immune response toward a Th1 type and away from a Th2 type of response, which would favor antibody production (31, 44). However, the delayed-type hypersensitivity response to picryl chloride, considered a Th1 response, in mice and the cytotoxic T lymphocyte response of NDV-infected chicks are both low. Conversely, specific antibody responses may not be decreased (as in the case of the elevated IgG2a response of vitamin A-deficient mice infected with influenza virus).

Evidence from in vitro experimentation with normal human lymphocytes supports a possible role for retinoic acid in the regulation of IL-2-mediated responses that are central to T cell-mediated immunity, antibody responses, and the activation of NK cells. The addition of retinoic acid to cultured human B cell lines or thymocytes led to an apparent inactivation of an inhibitor of transcription of the IL-2 receptor α subunit and an increase in the level of its mRNA (45, 46). On the basis of cell culture studies, it has also been proposed that retinoic acid down-regulates the transcription of the IL-2 gene (47). If retinoic acid functions to enhance expression of high-affinity IL-2 receptors while down-regulating IL-2 production, the result could be a more focused response limited to those cells expressing the high-affinity form of the IL-2 receptor, and therefore responsive to lower amounts of IL-2.

EFFECTS OF INFECTION AND INFLAMMATION ON RETINOL TRANSPORT AND METABOLISM

Vitamin A is transported from liver, its major site of storage, to target tissues in a complex consisting of one molecule of retinol bound noncovalently in the binding cavity of retinol binding protein (RBP), which in turn is associated noncovalently with transthyretin (48). The liver is the major site of synthesis and release of these relatively short-lived, homeostatically regulated transport proteins. Most, if not all, target organs receive retinol via this complex; in addition, plasma also contains retinoic acid at a lower concentration. Although the mechanism of retinol uptake by tissues is uncertain, there is reason to believe, based on studies in cultured cells, that the K_m for uptake (49) is similar to the normal plasma concentration (~1.5–3 μmol/l), such that delivery of the vitamin to tissues may be reduced even during moderate hyporetinemia. However, it is not yet known whether reductions in the concentration of retinol or RBP during inflammation (below) impair the delivery of retinol to target organs. The kidney is especially important with respect to the degradation of RBP and the recycling of retinol, which is known to recirculate between plasma and tissues several times before being degraded (48).

Evidence is increasing that several aspects of the transport and metabolism of retinol are altered during infection or experimental inflammation. During infections in children, plasma retinol concentrations may be low (as, for example, in respiratory syncytial virus infection; 50). In adults with pneumonia and sepsis, the urinary excretion of retinol and RBP, which normally is low, was elevated significantly, especially during fever (51); similarly, excretion was elevated in children with acute diarrhea (52). Thus, loss of retinol may be a mechanism contributing to the development of poor vitamin A status in young children or of clinical vitamin A deficiency if their vitamin A status already is poor. The inflammatory response to infection may also have a significant effect on the hepatic synthesis and secretion of RBP and, hence, on the delivery of retinol to target tissues. In normal, vitamin A-sufficient rats, the induction of inflammation by lipopolysaccharide caused a significant (~50%) reduction the plasma concentrations of retinol and RBP; these changes were preceded by a reduction in RBP mRNA in liver (53). These changes, which corresponded to the

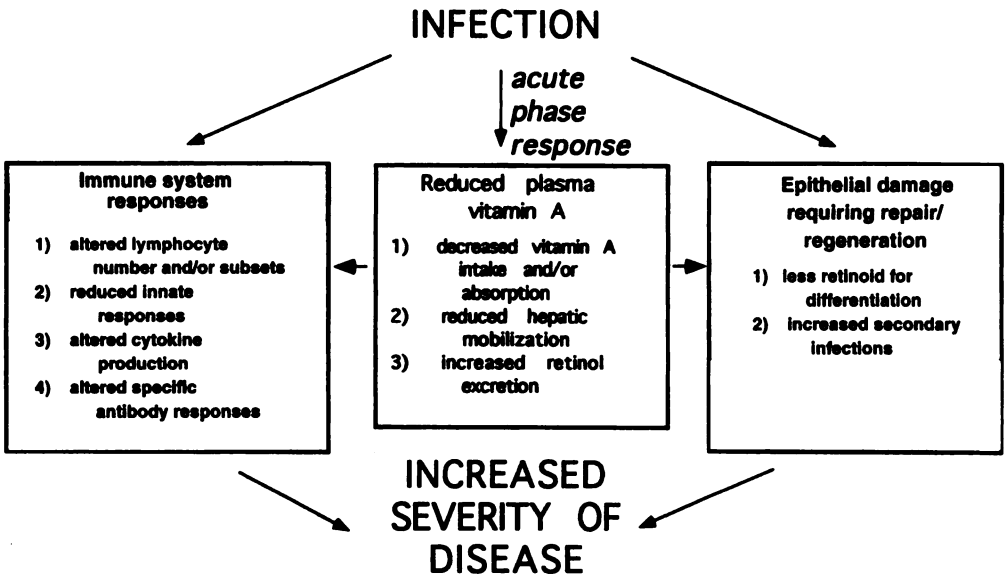

Figure 1. Model of the possible interactions between vitamin A status, infection and increased severity of infectious disease.

acute-phase response to infection, resulted in an acute hyporetinemia even though liver vitamin A reserves were more than adequate. The results of these studies imply that infection may induce changes in the transport and catabolism of vitamin A by reducing both the delivery of retinol from liver to tissues via RBP and the renal retention and recycling of retinol back to plasma.

POSSIBLE INTERACTIONS BETWEEN RETINOID METABOLISM AND THE SEVERITY OF INFECTION IN ANIMALS AND HUMANS

A conclusion drawn from the results of field-based epidemiologic studies is that the incidence or rate of infection does not differ between vitamin A-supplemented children and untreated controls. On the other hand, the data support a greater severity of diarrheal disease and measles in vitamin A-deficient vs. vitamin A-supplemented children. The lack of an effect of vitamin A on the incidence of infection has been interpreted as suggesting that preexisting differences in epithelial integrity are unlikely to be a main cause of the increased morbidity and mortality in vitamin A-deficient children. However, the results of animal studies discussed above, most notably with NDV and rotavirus, suggest that epithelial damage resulting from the infection is greater, and of longer duration, when vitamin A deficiency is also present. These results suggest that, although infection results in damage to the epithelia regardless of vitamin A status, a preexisting deficiency of vitamin A leads to a more profound and consequential impairment. At the same time, inflammation may reduce retinol transport both in individuals with normal vitamin A status and, we speculate, in those with impending vitamin A deficiency. In the former case, such an effect may be transient, but in the latter case the response to infection may precipitate a critical depletion of tissue retinoids. As noted previously, the foci of vitamin A-deficient epithelia may be sites of penetration of bacteria and other agents leading to secondary infections. Thus, as proposed in **Fig. 1**, vitamin A deficiency may result in a weak or aberrant immune response, whereas infection itself could play a twofold role by inducing epithelial damage and initiating an inflammatory response that further impairs retinol transport and/or increases catabolism. Derangements in immunity, retinoid metabolism, and tissue repair responses may interact, or even synergize, to increase the severity of infections in vitamin A-deficient animals and humans. FJ

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