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Review

Augmentation of antibody responses by retinoic acid and costimulatory molecules

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ABSTRACT

Antibody production is crucial for a successful vaccine response. Beyond the ability of vitamin A (VA) and its active metabolite, all-*trans*-retinoic acid (RA) to restore growth in VA-deficient animals, supplementation with VA and/or treatment with RA can augment antibody responses in both VA-deficient and VA-adequate animals. RA alone, and in combination with stimuli that are ligands for the Toll-like receptor family, can augment the adaptive immune response leading to a heightened primary antibody response, and a stronger recall response upon restimulation. Mechanisms may include regulation of cell populations, type 1/type 2 cytokines, and B cell-related transcription factors, leading to accelerated B cell maturation.

1. Introduction

Adaptive immunity is characterized by antigen (Ag) specificity, diversity, and immunologic memory, and usually results in more effective immune protection than from innate immunity alone. Understanding the nutritional–hormonal factors that regulate adaptive immune responses is highly important to human health. It is now well demonstrated that provision of an adequate intake of vitamin A (VA) to children aged 6 months to 5 years can reduce all-cause mortality by 23%, measles-related mortality by 50% [1], and diarrhea-related mortality by 23% [2,3]. The World Health Organization supports vitamin A supplementation as an important, cost-effective strategy to improve child health. For convenience and to reach more children, VA supplements are often delivered at vaccine clinics [1], and thus concurrent treatment with VA and vaccines has become a common practice.

Vitamin A (retinol) is an essential micronutrient required in the diet of all vertebrates [4]. VA is long been known as a requirement for the maintenance of normally differentiated epithelial cells, through the regulation of cell turnover, trafficking and mucin production. Thus, it is likely that some if not most of the clinical benefits attributed to VA supplementation are the result of its effects on the mucosal and systemic immune systems (see [5,6] for reviews of epidemiological studies). All-*trans*-retinoic acid (RA), one of two major metabolites of retinol, is a potent regulator of cell proliferation and differentiation [7], with pleiotropic effects, due to regulation of gene expression, in essentially all organ systems. The genomic actions of RA are mediated by its binding to transcription factors of the RA nuclear receptor family RAR (RARα, β and γ), while 9-*cis*-RA binds to RXR receptors [7,8]. RA may also have nongenomic activities through epigenetic mechanisms and via crosstalk with other signaling pathways [9].

2. Could RA be a "fourth signal" in the regulation of the antibody response?

Signals generated by the binding of Ag (signal 1) to its receptors, and costimulatory/accessory molecules (signal 2) to their respective receptors, are well known to be crucial for the development of Ab responses. "Danger signals" (signal 3) [10,11], now understood to be generated mainly by ligands for Toll-like receptors (TLR) [12], include biological agents such as lipopolysaccharide (LPS), a natural ligand for TLR4, and synthetic compounds such

Abbreviations: Ab, antibody; Ag, antigen; AID, activation-induced cytidine deaminase; APC, antigen-presenting cell; BCR, B cell antigen receptor; CSR, class switch recombination; DC, dendritic cell; GC, germinal center; GLT, germ line transcript(ion); Ig, immunoglobulin; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; NK, natural killer; NKT, natural killer T (cells); PMBC, peripheral blood mononuclear cells; PIC, polyriboinosinic:polyribocytidylic acid; RA, retinoic acid; RAR, retinoic acid receptor; RXR, retinoic X receptor; TD, T cell-dependent; Th, T-helper cell; TI, T cell-independent; TLR, Toll-like receptor(s); TT, tetanus toxoid; VA, vitamin A.

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as polyriboinosinic:polyribocytidylic acid (PIC), a ligand for TLR3 [12], or by cytokines produced by antigen-presenting cells (APC) or Ag-activated T cells. The small lipophile, RA, can also have a profound effect on the development, differentiation and immune outcome of B cells. In combination with stimuli like LPS and PIC, RA has the potential to induce a heightened primary response as well as a robust memory response [13,14]. RA may also accelerate the development of B cells, expanding or modifying the mature B cell pool available for stimulation [15]. The idea that RA can induce changes that result in a permanent commitment of immune system cells, or "imprint" them, was expressed by Iwata et al. regarding gut-homing T cells [16], but may be more generally applicable. The notion of RA as potentiating a strong and long-lived immune response is consistent with the well-established properties of RA as a powerful agent of cell differentiation [7]. Thus, RA could be an important "signal 4," when RA is present at an adequate concentration during the period of initial B cell and T cell stimulation. A signal from RA would be expected to act principally at the nuclear level, through ligation of RAR–RXR receptors, resulting in the induction or repression of critical genes that code for intracellular, plasma membrane-associated, or secreted proteins that, in turn, directly or indirectly modulate the immune response. A fourth signal delivered by RA could provide a final "imprint" or imprimatur on the cells involved, setting them on a course of progression towards a stronger, longer acting, or potentially altered response as compared to that in the absence of RA.

In this review we first discuss studies demonstrating the significance of adequate VA and RA for normal immunity and the ability of RA to augment the Ab response in vivo. We then discuss research using isolated cells that has begun to reveal pathways and processes that are modulated in the presence RA, including class switch recombination (CSR), proliferation, cytokine production, signaling, and B cell differentiation leading up to the formation of Ab-producing plasmacytes.

3. Vitamin A status, RA, and Ab titers in vivo

3.1. Vitamin A status as a factor in the in vivo antibody response

Studies published in the late 1980s and early 1990s revealed that Ab responses to Ag classified either as T cell-dependent (TD) and T cell-independent-type 2 (TI-2) are markedly reduced by VA deficiency (reviewed in [17]). The poor responses could be restored to normal levels relatively rapidly by treatment with VA, indicating the defect was due to a specific lack of this nutrient, while the reversibility also suggested that VA therapy would be useful in VA-deficient populations. In support of this, Semba et al. [18] showed that Indonesian children with xerophthalmia who were supplemented with VA produced a stronger anti-tetanus toxoid (TT) response than children not supplemented with VA. Surprisingly, animal studies designed to compare the response to different types of Ag in VA-deficient rats showed that the IgM response to TI-type 1 (LPS as Ag, given at low immunogenic doses with the Ab response measured as O-saccharide-specific IgM] was not reduced by VA deficiency [19]. Thus, the defect in Ab production was characterized as a dysregulation, in response to some types of Ag, rather than a complete inability to form a strong Ab response, and the requirement for VA for Ab production could therefore be described as *conditional*, dependent on the antigenic challenge. Although these results were obtained prior to discovery of the TLR family of receptors and an understanding of the ligands that activate them, the idea of "danger signals" had already been proposed [10,11], which suggested that microbial products like LPS might elicit cytokines or other factors that could have autocrine or paracrine effects on nearby cells. We therefore considered that coimmunization might effectively stimulate not only production of LPS-specific IgM, but also enhance the Ab response to a TD and TI-2 Ag, which otherwise was low in VA-deficient animals. Indeed, coimmunization *Pseudomonas aeruginosa* LPS (*Pa*-LPS) and with either pneumococcal polysaccharide, a TI-2 Ag, or TT, a TD Ag, resulted not only antibodies to Pa-LPS but a >3-fold stronger primary response to pneumococcal polysaccharide [19] or TT, both as IgM and IgG [20]. When TT (without LPS) was administered again to elicit a recall Ab response, the secondary anti-TT IgG response was 6–12 times higher compared to animals that had not been treated with LPS at priming [20]. In these studies LPS was not acting simply as a general B cell mitogen because, despite induction of Ag-specific Ab production, the total plasma Ab concentrations did not rise. These results showed that the potentiation of Ab production by LPS required its presence at the time of priming with the TI-2 or TD Ag, and also illustrated augmentation of class-switched memory response to a TD Ag such as TT. Moreover, similar results were observed in VA-adequate animals [21], suggesting the possibility that VA or RA combined with other stimuli might be an effective means to augment antibody responses even in a well-nourished population. With the great advancement that has taken place in knowledge about the TLR family and its ligands, these results now can be explained as being the result of a danger signal (signal 3) provided by LPS through its engagement with TLR4 on APCs, T cells, or B cells during their activation in response to immunization with TT, that significantly augmented both the primary Ab response and the formation of memory.

3.2. Interactions of RA and TLR pathway ligands

TLR ligands are now of great interest for their potential as vaccine adjuvants (reviewed in [22]), including natural compounds like LPS, a ligand for TLR4; synthetic ligands such as polyriboinosinic: polyribocytidylic acid (PIC), a dsRNA that mimics some of the effects of retroviruses and a ligand for TLR3; flagellin, a ligand for TLR5; and CpG, a mimetic of bacterial DNA and a ligand for TLR9 [12]. Several studies, summarized in Table 1, have demonstrated that RA can synergize with these signals to augment Ab production.

4. Possible mechanisms for augmentation of Ab responses in vivo

4.1. Altered cellularity

Normal VA status is required for maintaining the integrity of lymphoid organs and the balance of major lymphocyte populations. A lower proportion of CD4+ T cells and a lower CD4:CD8 T cell ratio have been noted in studies of children with xerophthalmia [25] as well as in rats fed a VA-marginal diet [26]. VA deficiency also diminished the number of splenic germinal centers (GC), total spleen cells, and spleen and thymus mass [27]. Conversely, RA increased the CD4:CD8 T cell ratio [14]. The effect of VA on APCs, and particularly dendritic cells (DC), has only recently begun to be elucidated, but evidence indicates that exposure to RA is critical for their functionality. Bone marrow cells cultured in low-VA medium with granulocyte-macrophage colony stimulating factor exhibited reduced DC differentiation, while granulocytes increased, while, conversely, repletion with RA significantly induced the formation of myeloid DC [28]. Furthermore, RA does-dependently increased the tumor necrosis factor-α-induced expression of major histocompatibility complex class II molecules and CD86 on immature Langerhans cell-type DC, suggesting enhanced DC maturation,