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VITAMIN D AND ITS ANALOGS AS REGULATORS OF IMMUNE ACTIVATION AND ANTIGEN PRESENTATION

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■ **Abstract** It has been a little more than 20 years since the first appreciation that the biologically active hormonal form of the secosteroid vitamin D—classically categorized as a regulator of calcium/phosphorous metabolism and bone mineralization—can exert effects on cells of the immune system. Since then a substantial literature has accumulated to suggest that these effects are exerted on multiple immune cell types, are predominantly suppressive at pharmacologic levels, and are potent enough to have true therapeutic potential in the management or prevention of immune-mediated diseases. Less clear at present, however, are the physiological roles played by the vitamin D endocrine system in the regulation of normal and abnormal immune responses. In this review, an appraisal of the current understanding of vitamin D–mediated immune regulation is presented that emphasizes progress towards its clinical application as well as the manner in which emerging models of normal immune function may facilitate a more complete understanding of its physiologic significance.

CONTENTS

THE IMMUNE SYSTEM AND ITS REGULATION: THE IMPORTANCE OF CROSS-TALK BETWEEN INNATE AND COGNATE IMMUNITY

The immune system has evolved primarily to provide rapid and effective protection against pathogenic microorganisms (63). Involving, as it does, an array of highly potent killing mechanisms, an essential component of normal immune function is the regulation of its responses in order to avoid self-injury (autoimmunity). Components of the immune system are often assigned to antigen-nonspecific (innate) or antigen-specific (cognate) categories. Innate responses to pathogens are virtually immediate, occur at the site of pathogen invasion, are based on fixed pattern recognition, and are amplified predominantly by the production of soluble (proinflammatory) products that stimulate or attract additional cell populations. Innate immunity is mediated by the parenchymal cells of all organs and tissues as well as by mobile cells with specialized functions including macrophages and natural killer (NK)[1] cells. In contrast, cognate responses occur following a priming period, are initiated in specialized organs distant from the disease site, are greatly diversified by post-transcriptional modifications, and are predominantly amplified by antigen-driven cellular proliferation. Cognate immunity is mediated by cells specialized for antigen presentation [dendritic cells (DCs)] and antigen recognition (T- and B-lymphocytes). Both T-cells and B-cells are capable of a number of different response patterns that ultimately dictate the nature and duration of immune activity (71). Coordinated regulation of innate and cognate arms of the immune system is essential both for the effective elimination of pathogens and for the

[1]Abbreviations are defined in Appendix.

prevention of self-destructive responses. A model of immune regulation is presented in Figure 1 that incorporates a number of emerging concepts and will serve as a template for discussing current understanding of the physiologic and therapeutic importance of vitamin D–mediated effects on the vertebrate immune system. Central to this model is the role of the DC (7, 49, 58, 90, 122). Dendritic cells have been shown to process protein antigens from invading microorganisms as well as from apoptotic or necrotic cells within peripheral tissues and to present these as major histocompatibility complex (MHC)/peptide complexes to T-cells in the spleen and lymph nodes (90). The outcome of antigen presentation by DCs is variable and is strongly dependent on the presence or absence of signals derived from the innate immune response (7, 49). Thus, self-antigen derived from apoptotic cells in the absence of innate signals may preferentially generate regulatory T-cells that actively suppress autoimmunity (58, 122). In contrast, antigen derived directly from invading microorganisms or from infected cells will be accompanied by an array of innate signals that instruct the DC to potently activate antigen-specific T- and B-cells (7, 49). Patterned T-cell responses (Th-1 and Th-2) are further selected for by antigen-bearing DCs through the secretion of modifying cytokines such as IL-12 (71). According to this model, therefore, both immune tolerance to self and immune activation against invading organisms are the result of similar antigen trafficking processes but differ in the context in which antigen is encountered. At all levels this cross-communication between the innate and cognate arms of the immune system is subject to positive and negative regulatory influences that may be tissue-specific or genetically variable. Furthermore, manipulations of innate/cognate interactions are likely to be of specific benefit in the prevention or treatment of diseases in which immune responses are deficient or maladaptive.

INITIAL OBSERVATIONS OF VITAMIN D IMMUNE EFFECTS

The Presence of Vitamin D Receptors in Cells of the Immune System

The first indication that receptors for the active form of vitamin D [1α,25-dihydroxycholecalciferol, subsequently referred to as 1,25$(OH)_2D_3$] are present in immune cells came from the observation of prodifferentiative effects of 1,25$(OH)_2D_3$ on murine myelomonocytic cell lines (1, 11, 60) and from biochemical studies demonstrating a vitamin D receptor (VDR) in human thymus and peripheral blood leukocytes (15, 104, 111). Subsequently, multiple authors were able to confirm that the VDR is constitutively expressed in significant amounts by human and murine monocytes and is inducibly expressed to similar levels in lymphocytes following delivery of activation signals (15, 82, 83, 102, 105). In lymphocyte-rich tissues such as tonsil and thymus, expression of VDR was confined to cells in the early stages of activation and mitogenesis (103, 107). As characterization of T-cell signaling requirements and subtypes improved, it was additionally shown that VDR

expression levels were highest following strong activation signals through the T-cell receptor (TCR) and accessory receptors (141) and were comparable in helper (CD4+) and cytotoxic (CD8+) subsets (102, 132).

Immune Consequences of Vitamin D Deficiency

In parallel with observations on VDR expression in immune cell subtypes, a smaller number of reports emerged suggesting immune defects in vitamin D–deficient patients and experimental animals (51). Most notably, studies by Bar-Shavit et al. (10) and Toss et al. (126) provided evidence for defects in macrophage proinflammatory function and in localized antigen-specific cellular immune responses [delayed-type hypersensitivity (DTH)] during vitamin D deficiency. More recently, Yang et al. (139) have definitively demonstrated reduced DTH responses in profoundly vitamin D–deficient mice. These findings, while consistent with a long-standing view that individuals with rickets are abnormally prone to infection (51), are paradoxical in the context of the abundant literature (reviewed in the following sections) supporting an immunosuppressive role for $1,25(OH)_2D_3$ and related analogs. Nonetheless, as subsequently discussed, a balanced model of the immunoregulatory effects of vitamin D must incorporate evidence that, under physiological conditions, $1,25(OH)_2D_3$ may function to facilitate rather than inhibit elements of the normal immune response.

THE EFFECTS OF VITAMIN D ON INDIVIDUAL IMMUNE CELL TYPES

Monocytes, Macrophages, and Dendritic Cells

At the time that receptors for $1,25(OH)_2D_3$ were identified in monocytes and myelomonocytic cell lines the monocyte was primarily viewed as a precursor form of the tissue macrophage—a phagocytic cell capable of secreting an array of proinflammatory products and intimately involved in host defense against bacterial infection. A number of studies demonstrated that $1,25(OH)_2D_3$ induced differentiation of monocytes and monocyte-derived cell lines toward a macrophage-like phenotype (11, 60, 101, 114, 137). Furthermore, investigations of patients with granulomatous conditions (sarcoidosis and tuberculosis) by Adams's and others' groups demonstrated the generation of $1,25(OH)_2D_3$ by disease-associated macrophages (4, 5, 8, 26). In contrast to hydroxylase regulation in the kidney, 25-hydroxyvitamin D_3 1α-hydroxylase activation in macrophages was not associated with induction of 25-hydroxyvitamin D_3 24-hydroxylase activity (4)—a phenomenon subsequently shown by Dusso et al. to be mediated by interferon-γ (47). The expression of 25-hydroxyvitamin D_3 1α-hydroxylase by macrophages has been formally confirmed by Overbergh et al. (96) and shown to be induced by activating stimuli (interferon-γ and LPS) or by viral infection (94, 96).

Combined with observations of increased infections and reduced capacity to mount inflammatory responses in the setting of vitamin D deficiency, these findings

suggest a paracrine role for $1,25(OH)_2D_3$ in promoting localized macrophage-dependent inflammation (4, 8, 26, 94, 114). In keeping with this model, pleural fluid from patients with tuberculous pneumonia was found to contain high concentrations of $1,25(OH)_2D_3$ (8). Studies of the antigen presentation function of monocytes yielded, however, qualitatively different results. A series of reports by Rigby et al. (117), Tsoukas et al. (128), and Xu et al. (137) demonstrated that monocytes exposed to $1,25(OH)_2D_3$ in culture have significant reductions in class II MHC expression and in T-cell stimulatory capacity. In addition, it was recognized that the ability to provide the accessory ("co-stimulatory") signals necessary for T-cell activation was also potently inhibited in $1,25(OH)_2D_3$-treated monocytes (116, 137).

A clearer understanding of these latter observations has been permitted by progress in two key areas of immunology: (*a*) the characterization of the specific co-stimulatory receptors expressed by antigen presenting cells (APCs), including CD80 (B7-1), CD86 (B7-2), and CD40; and (*b*) the identification of a specialized subtype of APC, also derived from monocytes, termed dendritic cells (DCs). Dendritic cells are now appreciated as the primary initiators of T-cell–mediated immune responses in vivo and, when fully mature, express high levels of class II MHC, CD80, CD86, CD40, and an array of other accessory ligands and immunostimulatory products (7, 90). Dendritic cells are present within essentially all organs and demonstrate migratory patterns that allow for the uptake and trafficking of disease-associated antigens from peripheral tissues to specialized lymphoid organs where cellular immune responses are initiated. The process by which DCs pass from low to high T-cell stimulatory capacity is termed "maturation" (7, 49, 90). Because inappropriate DC maturation may result in excessive or unnecessary T-cell–mediated immune responses (autoimmunity), this process is subject to multiple positive and negative regulatory influences. In addition, an active role for immature DCs in promotion of immune tolerance to self or non-threatening foreign antigens (such as gut flora or food components) has also been identified (58, 122). It is of considerable interest, therefore, that $1,25(OH)_2D_3$ has recently been shown by a number of groups to potently inhibit DC maturation by a VDR-dependent mechanism (13, 25, 27, 54, 55, 99, 100, 120, 130). These studies have been primarily carried out using DCs generated in culture from human monocytes or murine bone marrow–derived precursors and have demonstrated significant reductions in DC surface expression of class II MHC, co-stimulatory ligands (CD80, CD86, CD40), and other maturation-induced proteins (CD1a, CD83). An example of dose-dependent inhibition of DC maturation by a $1,25(OH)_2D_3$-derived analog is illustrated in Figure 2. This figure further demonstrates that the yield of DCs from bone marrow culture is potently inhibited by higher concentrations of $1,25(OH)_2D_3$ analog than those associated with impaired maturation (10^{-8} to 10^{-9} M versus 10^{-10} to 10^{-12} M). Secretion of the immunostimulatory cytokine IL-12 is also reduced in DCs generated in the presence of $1,25(OH)_2D_3$ while, in some but not all of these studies, DC secretion of the potentially immunosuppressive cytokine IL-10 was increased (99, 130). Functionally, the capacity of

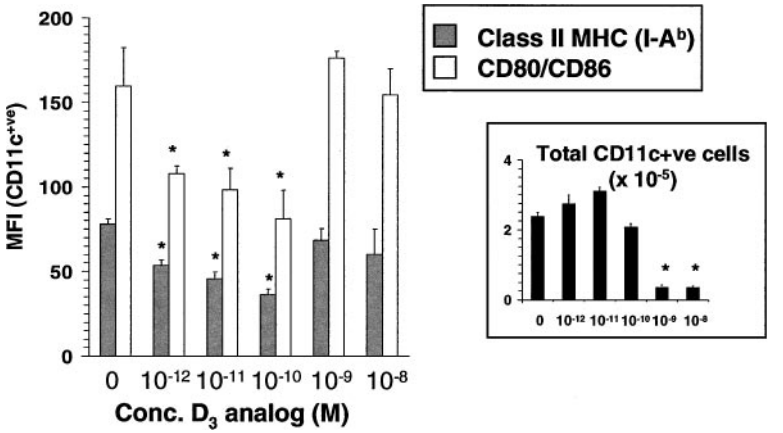

Figure 2 Biphasic inhibition of dendritic cell (DC) maturation by a $1,25(OH)_2D_3$ analog. Murine DCs were generated by culture of bone marrow cells with cytokines (GM-CSF + IL-4) for a total of seven days in the presence of varying concentrations (0 to 10^{-8} M) of the $1,25(OH)_2D_3$ analog $1,25(OH)_2$-16-ene-23-yne-26,27-hexafluoro-19-nor-D_3. Day-7 bone-marrow derived DCs were analyzed by flow cytometry for surface expression levels of class II MHC (I-A^b) and the co-stimulatory proteins CD80 and CD86. Results for these two markers of DC maturation are expressed as MFI of cells expressing the DC-specific cell surface protein CD11c (mean ± SD of triplicate wells for each concentration). Dose-dependent inhibition occurred up to 10^{-10} M D_3 analog. At concentrations greater than 10^{-10} M, DC surface levels of class II MHC and CD80/CD86 increased but significant inhibition of DC yield occurred (insert graph—total $CD11c^{+ve}$ cells per well shown as mean ± SD of triplicate wells for each concentration) revealing an inhibitory effect on the DC differentiation process at these high concentrations. * = $p < 0.05$ for values lower than corresponding results for cells not exposed to D_3 analog. *Abbreviations*: DC, dendritic cell; MHC, major histocompatibility complex; MFI, mean fluorescence intensity; SD, standard deviation.

$1,25(OH)_2D_3$-conditioned DCs to induce T-cell activation, proliferation, and cytokine secretion is profoundly impaired. The immature state of DCs generated in the presence of $1,25(OH)_2D_3$ is not readily reversed by typical maturing stimuli such as lipopolysaccharide or CD40 ligation and is persistent upon transfer of the cells in vivo as evidenced by the effect of inoculations of male $1,25(OH)_2D_3$ analog-conditioned DCs to promote tolerance in female experimental animals to subsequent male skin grafts (55). Although DCs have recently been shown to consist of a number of distinct subtypes in vivo, the specific effects of $1,25(OH)_2D_3$ on individual DC subtypes have not been reported to date. Nonetheless, with a growing appreciation of the potential for DCs as targets or vehicles for immune therapy, the recognition of the vitamin D_3/VDR pathway as a potent DC modulator is of clear clinical relevance.

T Lymphocytes

Direct inhibitory effects of $1,25(OH)_2D_3$ on T-cells have also been convincingly demonstrated although their physiologic and therapeutic importance relative to APC inhibition continues to be a source of conjecture. An extensive series of publications between 1984 and 1990 by the groups of Krane, Rigby, Manolagas, Lemire, and others established a basic profile for these effects (16, 17, 68, 81, 82, 112, 115, 118, 127). This included evidence that antigen- or lectin-stimulated human and murine T-cell proliferation, IL-2 secretion, and cell cycle progression from G_{1a} to G_{1b} are inhibited in vitro by concentrations of $1,25\ (OH)_2D_3$ between 10^{-12} and 10^{-8} M. The reported magnitude of this inhibition was quite variable and, in most studies, could be partially or completely overcome by IL-2 supplementation or application of more potent stimuli. Clarification of direct T-cell inhibition was provided by studies of highly purified T-cells and T-cell clones (70, 72, 76, 131). Although inhibition of IL-2 production has been repeatedly confirmed as a central mechanism for $1,25(OH)_2D_3$-mediated T-cell inhibition, more recent studies have also demonstrated IL-2-independent attenuation of interferon gamma (IFNγ), GM-CSF, and, perhaps, IL-4 production by T-cells (110, 113, 125). Despite the expression of VDR at comparable levels in both helper (CD4+) and cytotoxic (CD8+) T-cell subsets (102, 132), it has been repeatedly observed that CD4+ T-cells are the preferential target of $1,25(OH)_2D_3$-mediated inhibition during primary activation in vitro (73, 102, 131). Whether this preferential targeting of CD4+ T-cells also occurs in vivo or during secondary ("memory") activation of T-cells remains to be determined, although Meehan & DeLuca have observed that, in a murine model of demyelinating disease [experimental allergic encephalomyelitis (EAE)], the protective effect of $1,25(OH)_2D_3$ is preserved in mice lacking CD8+ T-cells (89).

The identification of CD4+ T-cell subsets with discrete cytokine secretion profiles [T-helper 1 (Th-1) and T-helper 2 (Th-2)] (71) as well the more recent identification of CD4+ T-cells with suppressor activity [regulatory T-cells (T_{reg})] (40) has stimulated additional interest in the T-cell modulatory effects of $1,25(OH)_2D_3$, particularly in the context of immune-mediated diseases. The polarization of activated CD4+ T-cells to a Th-1 phenotype (IL-2, IFNγ, TNFα secretion) or to a Th-2 phenotype (IL-4, 5, 13, 10 secretion) represents a major determinant of the nature of subsequent cellular and humoral immune responses (see Figure 1) and is a self-perpetuating process in that one subtype inhibits the generation of the other (71). The influence of $1,25(OH)_2D_3$ on T-helper polarization has been the topic of a number of recent reports (19, 64, 76, 121). Although some conflicting results have been presented, a balanced appraisal of the data from these studies suggests three specific conclusions: (*a*) the primary generation of Th-1-type T-cell responses is potently inhibited by $1,25(OH)_2D_3$ both in vitro and in vivo, (*b*) for T-cell activation occurring under conditions that are not strongly polarizing, the presence of supraphysiological concentrations of $1,25(OH)_2D_3$ will favor the emergence of a predominant Th-2 phenotype, (*c*) production of IFNγ

by previously activated T-cells is more potently inhibited than production of IL-4. Thus, while $1,25(OH)_2D_3$ does not directly up-regulate IL-4 and may, in fact, have modest inhibitory effects on the IL-4 promoter in naïve T-cells (121), its influence over T-helper polarization is likely to favor the Th-2 phenotype, particularly after repeated stimulations. In support of this conclusion, mice protected from EAE, murine lupus, and autoimmune diabetes by administration of $1,25(OH)_2D_3$ have evidence of Th-2 polarization in vivo (34, 46, 97).

A separate and provocative possibility—that $1,25(OH)_2D_3$ may promote the generation of regulatory T-cells (T_{reg})—has also been raised (52, 53, 88). The characteristic features of T_{reg}, as defined by a burgeoning literature, include the combined expression of CD4 and CD25, the secretion of potentially inhibitory cytokines (IL-10 and TFG-β), and the ability to potently inhibit antigen-specific T-cell activation (40). The fact that T_{reg} have been reported by different groups to mediate inhibition by both contact-dependent and noncontact-dependent mechanisms suggests that more than one population of such cells exists (40). In a murine islet cell transplant model, Gregori et al. (52) found that the combination of $1,25(OH)_2D_3$ and the immunosuppressant drug mycophenolate mofetil not only protected against graft rejection, but increased the proportion of CD4+CD25+ T-cells in regional lymph nodes. Furthermore, transfer of these cells to untreated animals resulted in protection from rejection—a characteristic feature of T_{reg} (52). Similar observations have been made by the same group in a model of autoimmune diabetes (53). In this model, treatment with a $1,25(OH)_2D_3$ analog alone was sufficient to enhance protective T_{reg} activity. The authors speculate that the promotion of T_{reg} by $1,25(OH)_2D_3$ represents the indirect result of DC modulation rather than a direct T-cell effect. In an APC-free in vitro system, however, Barrat et al. (9) demonstrated that repeated stimulation of CD4+ T-cells in the presence of $1,25(OH)_2D_3$ and a corticosteroid (dexamethasone) resulted in a homogenous population of cells with predominant IL-10 production and the ability to suppress autoimmune demyelination in vivo in an antigen-specific manner. Thus, although the role of $1,25(OH)_2D_3$ in the normal generation and maintenance of T_{reg} and the promotion of self-tolerance remains speculative, the combination of $1,25(OH)_2D_3$ agonists with additional immunomodulatory agents (as expanded upon below) may hold specific promise in this regard.

B Lymphocytes and NK Cells

The influence of $1,25(OH)_2D_3$ on other major immune cell subtypes—antibody-producing B-cells and plasma cells or natural killer (NK) cells—has been only poorly defined. Although earlier studies reported B-cells among the cell types expressing a receptor for $1,25(OH)_2D_3$ (104), a more recent analysis by Veldman et al. (132) failed to detect significant VDR expression in resting or activated B-cells. Functionally, an inhibition of mitogen- or antigen-stimulated immunoglobulin production (IgM and IgG) by $1,25(OH)_2D_3$ agonists has been observed by a number of authors in vitro and in vivo (41, 62, 67, 74, 138). In most of these

experiments it was not possible to distinguish between indirect effects on T-cell help and a direct effect on B-cells. In one report by Provvedini et al. (105), however, T-cell-independent inhibition of stimulated immunoglobulin production by Epstein Barr Virus-transformed human B-cells was shown. In this same study, expression of receptor for $1,25(OH)_2D_3$ by the transformed B-cells was detected raising the possibility that B-cells may be induced to respond to $1,25(OH)_2D_3$, under specific circumstances. Finally, in an in vivo study, Abe et al. (3) described an enhancement of antisheep erythrocyte antibody production following treatment with a low dose of a $1,25(OH)_2D_3$ analog. Based on this scant literature it is difficult at present to estimate the potential for $1,25(OH)_2D_3$ agonists to modify antibody-mediated disease processes. For NK cells, a lymphocyte subset involved in lysis of disease-associated cells that demonstrate altered expression of class I MHC and other normal surface proteins, even less information is available regarding the effect, if any, of $1,25(OH)_2D_3$. In a single report, the generation of "NK activity" in human peripheral blood mononuclear cell cultures was attenuated by addition of $1,25(OH)_2D_3$ but the cytotoxicity of NK cells was not directly inhibited (91). Thus, there is currently no convincing evidence for a direct interaction between this cell type and the vitamin D system.

VITAMIN D–MEDIATED MODULATION OF INTRACELLULAR SIGNALING IN IMMUNE CELLS

Activation events in lymphocytes and APC populations are the result of altered gene expression patterns that are orchestrated by an array of intracellular signaling pathways. The signaling mechanisms operational in lymphocytes and APCs are, in some cases, unique to cells of the immune system but, in the majority, are shared by many cell types. The mechanism of action of most immunomodulatory agents can be linked to discrete effects on one or more such pathways. Although the primary mechanism of action of $1,25(OH)_2D_3$ is to directly regulate the transcription of individual genes by forming a DNA-binding complex with the VDR and other associated proteins (35, 66), there is abundant evidence that many of its effects on cellular function result indirectly from modulation of intracellular signaling pathways. Although it is beyond the scope of this review to document in detail the reported signaling modifications that have been ascribed to $1,25(OH)_2D_3$, there have been a variety of studies demonstrating potent effects of $1,25(OH)_2D_3$ on some of the signaling pathways that are integral to the regulation of antigen presentation and lymphocyte activation (6, 42, 45, 59, 84, 123, 136, 140). These findings are summarized in Table 1. An example of $1,25(OH)_2D_3$-mediated modulation of a key intracellular signaling pathway involved in immune function (the NF-κB pathway) is provided in Figure 3. In this experiment an additive effect of the combination of a $1,25(OH)_2D_3$ agonist and a corticosteroid is also shown. The NF-κB pathway consists of a number of related proteins that regulate gene expression when translocated to the nucleus as hetero- or homodimers and is central to differentiation, maturation, activation, and survival of APCs,

TABLE 1 Signaling pathways modulated by $1,25(OH)_2D_3$ in lymphocytes and antigen presenting cells

Signaling pathway modulated by $1,25(OH)_2D_3$	Cell types identified	Mechanism(s) of signaling pathway modulation	Target genes	Refs
Nuclear factor kappa B (NF-κB)	T-cells Dendritic cells	Inhibition of total intracellular levels of multiple NF-κB proteins	p105/p50 c-Rel Rel-B	140, 136
	Dendritic cells	Indirect inhibition of NF-κB heterodimer binding to promoter site	IL-12 p40 (?IL-12 p35)	45
Nuclear factor of activated T-cells (NFAT)	Activated T-cells	Direct DNA binding of VDR/RXR and interference with NFATp/AP-1 complex assembly	IL-2 (?GM-CSF, IL-4, TNFα)	6, 123
Phosphatidylinositol-3 kinase (PI3-K)	Promonocytic cell lines Monocytes	Activation of PI3-K activity by formation of a $1,25(OH)_2D_3$-dependent VDR/PI3-K complex	CD14, CD11b	59
Mitogen activated protein kinase (MAPK) pathways	Promonocytic cell lines	Activation of erk1/erk2 kinase	CD14, CD11b	84
	T-cell hybridoma	Indirect inhibition of c-myc-mediated transcriptional activity	Fas ligand	42

lymphocytes, and other cells of the immune system (50). Furthermore, there is good evidence for specialized roles of individual NF-κB proteins in different aspects of immune regulation (44, 56, 93). The discrete modification of intracellular levels or function of NF-κB proteins such as Rel-B and c-Rel by $1,25(OH)_2D_3$ alone or in combination with other immunosuppressants is likely to underlie a number of the immunomodulatory effects of $1,25(OH)_2D_3$ including IL-12 secretion, upregulation of MHC and co-stimulatory ligands in response to innate immune signals, and T-cell proliferation and survival (45, 136, 140). Finally, there are convincing examples of $1,25(OH)_2D_3$-dependent binding of VDR complexes directly to promoter regions of activation-induced genes in T lymphocytes. As an example, Cippitelli & Santoni (43) have observed that inhibition of human IFNγ promoter activity is associated with specific affinity of VDR and retinoic X receptor (RXR) for two separate regions of the promoter. The mechanism of transcriptional inhibition following DNA binding was not identified but may include interference with the recruitment of additional transcription factors such as AP-1 or a direct interference with the transcriptional machinery (43). It is very likely that additional molecular mechanisms, both nuclear and cytoplasmic, will be identified as playing a role in the immunomodulatory effects of $1,25(OH)_2D_3$ and related $1,25(OH)_2D_3$ analogs.

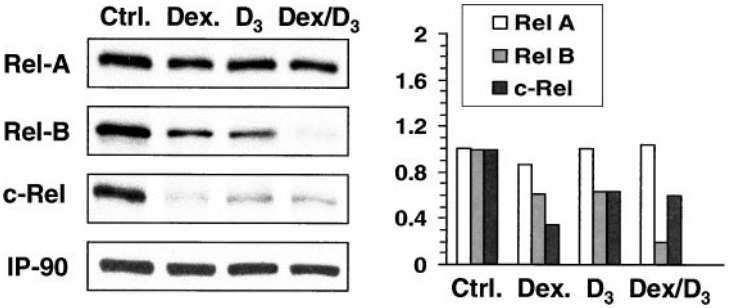

Figure 3 Discrete modulation of intracellular levels of NF-κB signaling proteins by a $1,25(OH)_2D_3$ analog and dexamethasone. Total cell lysates from control murine (Ctrl) dendritic cells (DCs) or DCs generated in the presence of optimized concentration of a $1,25(OH)_2D_3$ analog (D_3), the corticosteroid dexamethasone (Dex), or the two agents combined (Dex/D_3) were examined by Western blotting for the presence of three members of the NF-κB family of signaling proteins—Rel-A, Rel-B, and c-Rel—as well as the control protein IP-90. Quantification of relative expression levels of each protein in the four DC populations by densitometic analysis is shown in graphical form in the right panel. Results are expressed as relative density compared to that of control cells and are normalized for expression of IP-90. The results illustrate that expression of Rel-B and c-Rel but not Rel-A was inhibited by treatment of DCs with $1,25(OH)_2D_3$ analog as well as by dexamethasone. When combined, the two steroid agonists exerted a striking additive inhibitory effect on expression of Rel-B—a protein known to play a central role in DC differentiation and maturation.

THE EFFECTS OF VITAMIN D THERAPY ON EXPERIMENTAL MODELS OF IMMUNE-MEDIATED DISEASE

A considerable number of animal studies have been carried out to examine the effects of $1,25(OH)_2D_3$ and related analogs on immune-mediated diseases. These disease models, the great majority of which have been developed in the mouse and rat, serve the important function of allowing the therapeutic potential of $1,25(OH)_2D_3$ agonists in analogous human diseases to be tested. In addition, they have facilitated the ongoing investigation of the mechanisms of action of $1,25(OH)_2D_3$ within the intact immune system in vivo. Finally, with the development of a large number of $1,25(OH)_2D_3$-related analogs, the use of animal disease models provides an indispensable tool in which to determine relative efficacy and toxicity of such compounds compared to the parent compound. The disease models that have been employed to study $1,25(OH)_2D_3$ immunomodulation can be considered under three categories:

1) *Genetically Based Autoimmune Disease*: Many, if not all, human autoimmune diseases have an important genetic component. The influence of single

and multiple genetic polymorphisms on the development of autoimmunity has been greatly advanced by the recognition of naturally arising rodent strains and genetically modified murine lines with predisposition to specific autoimmune disease. Two such models have been applied to the study of $1,25(OH)_2D_3$-mediated immunotherapy. The first of these, the $MRL^{lpr/lpr}$ murine strain, represents a model of systemic lupus erythematosis (SLE) stemming from a single genetic mutation that results in the inactivation of the Fas apoptotic pathway. Among the manifestations of systemic autoimmunity in these mice are generalized lymphoproliferation, immune-complex-mediated nephritis, polyarthritis, skin disease, and the generation of anti-DNA antibodies (95). Studies by Koizumi et al. (69) and Abe et al. (2) in which $MRL^{lpr/lpr}$ mice were continuously treated with analogs of $1,25(OH)_2D_3$ from five or six weeks of age demonstrated partial reversal of autoimmunity in treated animals with reduced lymphoproliferation, nephritis, arthritis and prolonged survival but no effect on production of autoantibodies. Lemire examined the effects of a nonhypercalcemic dose of $1,25(OH)_2D_3$ on $MRL^{lpr/lpr}$ mice and noted improvement in nephritis and skin lesions but not generalized lymphoproliferation (79). Together these studies suggest a partial beneficial effect of $1,25(OH)_2D_3$ agonists on SLE-like disease when initiated prior to the onset of clinical manifestations.

A second model in which the therapeutic and mechanistic effects of $1,25(OH)_2D_3$ have been extensively studied is the nonobese diabetic (NOD) mouse, a strain in which multiple genetic polymorphisms lead to a high incidence of autoimmune diabetes mellitus (135). In this model, many of the immune phenomena that have been observed are similar to those that have been characterized in human type I diabetes mellitus (135). The group of Mathieu and Bouillon and colleagues have clearly shown that treatment of NOD mice with $1,25(OH)_2D_3$ or a number of related analogs prior to onset of diabetes can considerably reduce the subsequent development of pancreatic islet immune infiltration (insulitis) and overt diabetes without inducing broad immunosuppression (85). This finding has been recently confirmed by Gregori et al. using a nonhypercalcemic $1,25(OH)_2D_3$ analog (53). Bouillon and colleagues have also examined the therapeutic efficacy of $1,25(OH)_2D_3$ agonists administered after the development of insulitis and in diabetic NOD mice receiving syngeneic islet transplants in whom recurrent autoimmunity is invariable (37, 39). The reported results suggest that introduction of a $1,25(OH)_2D_3$ analog in animals with an established autoimmune process exerts only a minor effect on islet destruction. In contrast, the combination of a $1,25(OH)_2D_3$ analog with additional immunomodulatory agents (cyclosporine or interferon β) could significantly retard the progression or recurrence of the autoimmune response to insulin-producing cells (37, 39, 57). This same group, as well as that of Adorini, has examined a number of the mechanistic aspects of $1,25(OH)_2D_3$-mediated protection against autoimmune diabetes in NOD mice (36, 38, 53, 97). These studies

reveal a complexity that is unlikely to be explained by a single cellular target or inhibitory mechanism. Included among the important observations from these investigations is evidence for in vivo suppression of Th-1 T-cell responses, a permissive effect on autoantigen-specific Th-2 T-cell responses, development of autoantigen-specific regulatory T-cells, and restoration of activation- or drug-induced apoptosis in thymocytes and effector T-cells from NOD mice. Combined influences of $1,25(OH)_2D_3$ agonists on APCs and activated T-cells are likely to underlie such observations.

2) *Induced Immune-mediated Disease*: Many animal models of induced autoimmunity and immune-mediated tissue injury have been developed with variable degrees of similarity to human diseases. In contrast to the genetically based models, these induced models provide an opportunity to study the efficacy of therapeutic intervention at precisely defined points during a disease process in previously healthy adult animals. An array of such models have been applied to the study of $1,25(OH)_2D_3$ immunotherapy either alone or in combination with other immunosuppressants. Among the individual disease models for which some degree of efficacy has been shown for $1,25(OH)_2D_3$ agonists are mercuric chloride-induced nephritis (80), Heymann nephritis (22), anti-Thy-1.1 mesangial proliferative nephritis (98), Lyme arthritis and collagen-induced arthritis (30). In these models, the effects of $1,25(OH)_2D_3$ or related analogs could generally be characterized as a partial inhibition of disease phenotype when therapy was initiated before or at the time of the induction protocol. In the case of collagen-induced arthritis, however, complete prevention of disease or treatment of established disease could be achieved with higher doses of $1,25(OH)_2D_3$ therapy (30). For mercuric chloride-induced nephritis (80) and another model, autoimmune thyroiditis (48), an additive therapeutic benefit could be demonstrated for $1,25(OH)_2D_3$ agonists and cyclosporine.

One murine model of induced autoimmunity, experimental allergic encephalomyelitis (EAE), merits specific review. Inoculation of susceptible strains of mice with spinal cord homogenate, myelin basic protein (MBP), or MBP-derived peptides along with an immune adjuvant results in the development of a relapsing and remitting demyelinating disorder closely resembling the human disease multiple sclerosis (92). The immune mechanisms underlying this disease model have been extensively characterized and include generation of a predominantly Th-1-type cellular immune response (87, 92). Significant therapeutic effects of $1,25(OH)_2D_3$ or $1,25(OH)_2D_3$ analogs have been reported in EAE by Lemire & Archer (75), Cantorna et al. (29), Branisteanu et al. (24), and Mattner et al. (87). In the first of these reports by Lemire, parenteral administration of 100 ng of $1,25(OH)_2D_3$ every other day from 3 days before to 15 days after inoculation with spinal cord homogenate resulted in decreased disease activity and anti-MBP antibody formation as well as increased survival compared with controls (75). Cantorna et al. subsequently showed that daily oral supplementation with

20 ng of $1,25(OH)_2D_3$ was sufficient to prevent severe primary demyelination. Furthermore, later introduction of $1,25(OH)_2D_3$ therapy could halt established EAE progression although this effect was reversible upon treatment withdrawal (29). Similar findings were reported by Mattner et al. using a $1,25(OH)_2D_3$ analog with significantly lower capacity to induce hypercalcemia (87). Branisteanu et al. have shown clear beneficial effects in EAE by combining subtherapeutic dosages of $1,25(OH)_2D_3$ and the immunosuppressants cyclosporine (24) and sirolimus (rapamycin) (23). The mechanism of action of $1,25(OH)_2D_3$ in EAE has been the subject of a number of studies by these same groups although in some respects the results have been inconsistent. Evidence for a deviation toward a Th-2 T-cell response to MBP has been presented by Cantorna et al. in experiments showing increased IL-4 and TGF-β transcripts in the brains of $1,25(OH)_2D_3$-treated mice (34) and reduced therapeutic efficacy of $1,25(OH)_2D_3$ in EAE-prone IL-4 knockout mice (33). In contrast, Mattner et al. report a lack of effect of $1,25(OH)_2D_3$ analog therapy on IL-4 production by disease-specific T-cells but a potent inhibition of Th-1 (interferon-γ) responses (87). Thus, while the therapeutic potential for $1,25(OH)_2D_3$ agonists in immune-mediated demyelinating disease is evident, the mechanistic basis for this beneficial effect in vivo remains unclear.

3) *Transplantation*: The success of organ and cellular transplantation is highly dependent on the use of immune suppression, and animal models of transplantation represent an essential testing ground for novel immunosuppressive agents. Major goals in the advancement of transplantation medicine include the reduction of long-term toxicity associated with immunosuppressive regimens and the identification of agents that promote immune tolerance to grafted tissues without compromising overall protective immunity. For both of these goals there is evidence that $1,25(OH)_2D_3$ agonists may be of significant benefit in posttransplant immunosuppression. The efficacy and toxicity of $1,25(OH)_2D_3$ and related analogs has been studied in a variety of mouse and rat transplant models including skin (134), heart (61, 65, 77), kidney (108), intestine (65), liver (109), pancreatic islets (52), and aorta (106). As monotherapy, these agents have typically been shown to result in significant but modest prolongation of graft function. More strikingly, the combination of a $1,25(OH)_2D_3$ agonist with cyclosporine or mycophenolate mofetil—both immunosuppressants in common clinical use for transplantation—has been observed to result in prolonged graft survival compared with either agent alone (52, 108, 134). Furthermore, the benefits of $1,25(OH)_2D_3$ therapy following transplantation can accrue with limited detrimental effects on bone density and resistance to infection (31). Of additional interest, attenuation of vascular intimal proliferation and thickening has been reported in a rat aortic allograft model following combined treatment with the $1,25(OH)_2D_3$ analog MC1288 and cyclosporine (106). This finding suggests a potential benefit in the vascular injury that is common to various organ allografts

undergoing chronic rejection. As previously discussed, the work of Gregori et al. in a pancreatic islet model provides evidence that the combination of $1,25(OH)_2D_3$ with mycophenolate mofetil may promote graft-specific immune tolerance by inducing or permitting the development of regulatory T-cells (52). Taken together, these studies of animal allografts provide a basis for the testing of $1,25(OH)_2D_3$ analogs in human transplantation.

TOWARD SUCCESSFUL CLINICAL APPLICATION OF VITAMIN D–MEDIATED IMMUNOMODULATION: THERAPEUTIC CONSIDERATIONS

Despite the abundance of in vivo data that has been summarized to this point, the use $1,25(OH)_2D_3$ agonists for the treatment of immune-mediated disease has been limited to topical application of the $1,25(OH)_2D_3$ analog calcipotriol in psoriasis. Undoubtedly, the primary reason for this delay in clinical application lies with the systemic toxicity of $1,25(OH)_2D_3$ itself. There is now, however, considerable optimism that this hurdle will be overcome by the use of modified analogs of $1,25(OH)_2D_3$ with favorably altered in vivo activity. In this regard, it is important to examine the degree to which animal model experiments provide support for the design of clinical trials of $1,25(OH)_2D_3$ analogs in human immune-mediated diseases. A number of specific areas should be considered when evaluating potential immunotherapeutic applications of individual $1,25(OH)_2D_3$ analogs.

In Vitro Versus In Vivo Potency

The systemic bioavailability of $1,25(OH)_2D_3$ in vivo is dependent on its overall concentration in the serum and, perhaps, on the concentration of vitamin D–binding protein (DBP) (21, 66). The magnitude of its effect upon a target tissue is, furthermore, dependent on intracellular levels of vitamin D receptor (VDR), on the concomitant expression of additional members of the DNA-binding complex such as retinoic X receptor (RXR), and on the rate of conversion of $1,25(OH)_2D_3$ to inactive metabolites (21, 35, 66). Analogs of $1,25(OH)_2D_3$ may differ considerably from the parent compound in bioavailability and target tissue potency on the basis of alterations in affinity for DBP, alterations in susceptibility to degradation, and alterations in affinity for or conformational modification of the VDR (20). These in vivo complexities should be appreciated when interpreting $1,25(OH)_2D_3$- and $1,25(OH)_2D_3$ analog-mediated effects in in vitro culture systems. Although the presence of serum in most culture systems represents a source of DBP, concentrations may vary considerably. In addition, the induction of degrading enzymes in cultured cells may not mimic that to be encountered in whole organs or tissues in vivo. As demonstrated by Bouillon (20), analogs with low affinity for DBP may demonstrate enhanced in vitro potency compared to $1,25(OH)_2D_3$ but may prove significantly less potent in vivo if subject to rapid intracellular metabolism. The combination of partial DBP affinity, attenuated susceptibility to metabolic inactivation,

and preserved affinity for VDR would appear to identify analogs with high in vivo potency. For the purpose of human immunotherapy, however, a $1,25(OH)_2D_3$ analog must demonstrate a further distinction from the parent compound—a separation between in vivo immunomodulatory and hypercalcemic potency.

Hypercalcemia

The primary toxicity of $1,25(OH)_2D_3$ is the induction of hypercalcemia as a result of enhanced intestinal absorption and bone calcium mobilization (66). It is clear from many studies involving animal models of immune-mediated disease that the efficacy of $1,25(OH)_2D_3$ therapy is limited by the occurrence of hypercalcemia with increased or prolonged dosage (79, 80, 138). The capacity for various $1,25(OH)_2D_3$ analogs to induce hypercalcemia or to reduce bone calcium content has been studied by many groups. Although it is likely that all $1,25(OH)_2D_3$ analogs retain the tendency to increase serum calcium if administered at sufficient doses (22, 77, 80, 134, 138), it is clear from a number of recent studies that some immunomodulatory analogs are many-fold less potent in this regard than the parent compound (2, 18, 21, 37, 53, 57, 78, 142, 143). The mechanistic basis for this reduced hypercalcemic effect has not been completely elucidated but it is possible that, in addition to possessing modifications that favor high intracellular accumulation, such analogs induce conformational changes in the VDR that alter the structural and functional properties of the entire DNA-binding complex (133). In this way it has been proposed that $1,25(OH)_2D_3$ analogs, when bound to VDR, are responsible for modified gene expression profiles compared to the physiologic $1,25(OH)_2D_3$/VDR complex (35, 133). Further characterization of the molecular events underlying the separation of hypercalcemic and nonhypercalcemic effects of $1,25(OH)_2D_3$ agonists may allow for the development of even more selective analogs as immunotherapeutic agents.

Timing and Duration of Therapy

The optimal timing and duration of therapy with $1,25(OH)_2D_3$ or $1,25(OH)_2D_3$ analogs should be carefully examined when evaluating the potential for translating experimental results into human clinical trials. Some of the in vivo studies in which $1,25(OH)_2D_3$ or related analogs were found to favorably alter immune responses report relatively narrow therapeutic dose ranges (2, 53, 75, 79). In some studies, small variations in dosage or dose frequency were found to be associated with loss of efficacy or emergence of toxicity (53, 75, 79). In others, loss of efficacy was observed with dose levels both above and below the optimal dose (2). It is interesting to compare these observations with a number of in vitro phenomena in which the titration of $1,25(OH)_2D_3$ agonists across a range of concentrations has resulted in biphasic responses (54, 112). An implication of these results is that careful preclinical studies must be carried for $1,25(OH)_2D_3$ analogs to identify dosages with both minimal toxicity and optimal immunomodulatory effects.

In general, initiation of $1,25(OH)_2D_3$ therapy prior to or coincident with the induction of disease has been associated with the most striking beneficial effects

for models of immune-mediated diseases. Furthermore, disease progression or relapse has been observed when therapy has been withdrawn (29). This implies that the clinical use of $1,25(OH)_2D_3$ agonists could be best applied to disease prevention (such as in transplantation or prediabetic patients) or the maintenance of remission in relapsing conditions (such as multiple sclerosis). There are, however, examples of models in which significant improvements in an established autoimmune process have been reported. In these experiments, higher doses and additional manipulations to avoid hypercalcemia have been required (29, 30). These observations suggest that $1,25(OH)_2D_3$ analogs with reduced toxicity may also prove to be effective, either alone or in combination with other immunomodulatory agents (37, 39), in managing active immune diseases. Although the majority of animal studies in which duration of therapy has been examined suggest that continuous, long-term therapy with a $1,25(OH)_2D_3$ agonist will be required to prevent disease relapse, Adorini's group has observed long-lived protection against islet allograft rejection and autoimmune diabetes following limited courses of therapy (52, 53). These experiments raise the possibility that manipulation of the immune system by $1,25(OH)_2D_3$ may favor the emergence of persistent immune regulatory mechanisms.

Combined Effects With Other Immunomodulatory Agents

The effect of combining $1,25(OH)_2D_3$ or related analogs with other immunomodulatory agents has been referred to repeatedly in preceding sections and many studies have confirmed additive or synergistic effects in vitro and in vivo using such combinations. Among the clinically relevant agents that have been reported to enhance the immunosuppressive effects of $1,25(OH)_2D_3$ agonists are corticosteroids (14, 64, 69, 136), the calcineurin inhibitors cyclosporine and tacrolimus (24, 37, 48, 80, 108, 129, 134), the cell cycle inhibitor sirolimus (23, 129), the antiproliferative agents mycophenolate mofetil (52, 129) and leflunamide (129), and the cytokine interferon-β (57). In the case of corticosteroids, we have recently reported a potent additive effect of a $1,25(OH)_2D_3$ analog with dexamethasone on the ability of DCs to stimulate T-cell activation (136). This finding is demonstrated in Figure 4. As shown, the ability of bone marrow-derived DCs to potently stimulate the proliferation of antigen-specific CD4+ T-cells is significantly inhibited by generation of the DCs in the presence of $1,25(OH)_2D_3$ or corticosteroid agonists alone and is virtually abolished by the combined effects of the two steroid hormone pathways. In a detailed analysis, Van Etten et al. (129) have directly compared the synergism between $1,25(OH)_2D_3$ or $1,25(OH)_2D_3$ analogs and a panel of immunosuppressants in human T-cell cultures and in the murine EAE model. Their results confirm the in vitro and in vivo synergy between the $1,25(OH)_2D_3$ agonists and all of the immunosuppressants tested but reveal that synergy is most potent with the calcineurin inhibitors and sirolimus. It is highly likely, therefore, that clinical regimens combining $1,25(OH)_2D_3$ analogs with one or more of the immunosuppressants in current clinical use can

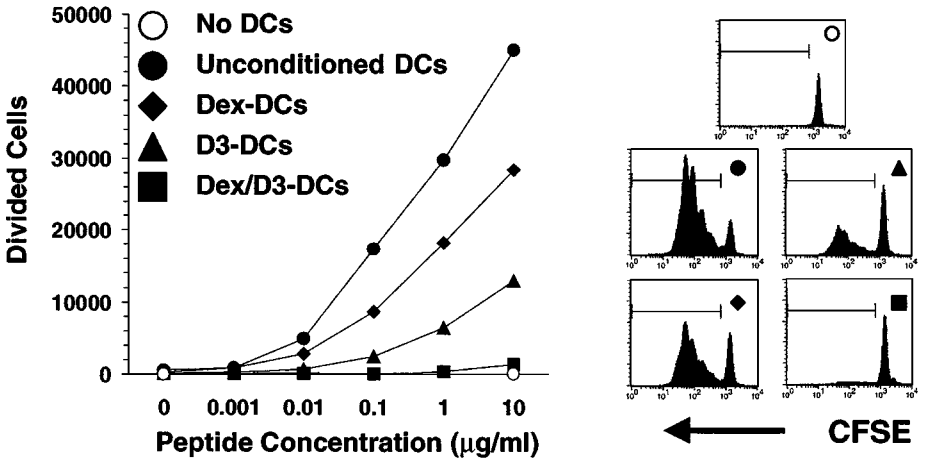

Figure 4 Combined inhibition of antigen presenting function of dendritic cells (DCs) by $1,25(OH)_2D_3$ analog and the corticosteroid dexamethasone. Murine CD4+ T-cells expressing a transgenic T-cell receptor specific for a peptide derived from ovalbumin were cocultured with four populations of bone marrow-derived DCs in the presence of a graded concentration of antigenic-peptide (0 to 10 μg/ml). The DC populations used were unconditioned or were cultured in the presence of optimized concentrations of the corticosteroid dexamethasone (Dex-DCs), of $1,25(OH)_2D_3$ analog (D3-DCs), or of the two agents combined (Dex/D3-DCs). T-cell proliferation was detected by labeling the cells with the fluorescent dye CFSE, which is sequentially diluted with each cell division and can be analyzed by flow cytometric analysis (*right panel*). Results, following 72 hours of culture, are expressed graphically as number of divided cells per sample (*left panel*) and demonstrate that the DC-induced, peptide concentration-dependent proliferation of CD4+ T-cells is inhibited to a greater extent by $1,25(OH)_2D_3$ analog conditioning than by dexamethasone conditioning and is essentially eliminated by generation of DCs in the presence of both agents.

be designed to both enhance the efficacy and reduce the toxicity of each agent.

Dietary Influences

A final consideration in optimizing the effectiveness of $1,25(OH)_2D_3$ analogs in immune-mediated diseases has been raised by the observations of DeLuca and Cantorna on the role of dietary calcium content in a number of murine models (32, 46). These authors have reported that the beneficial effects of $1,25(OH)_2D_3$ in EAE- and SLE-prone mice are significantly attenuated by a low calcium intake. Interestingly, the induction of hypercalcemia during $1,25(OH)_2D_3$ therapy did not restore therapeutic efficacy in animals on low calcium diets. Although the mechanism underlying such an effect is unclear, the results suggest that restriction of dietary calcium in patients receiving $1,25(OH)_2D_3$ analogs may actually limit therapeutic efficacy.

TOWARD AN UNDERSTANDING OF THE PHYSIOLOGIC ROLES OF VITAMIN D IN IMMUNE FUNCTION

The extensive literature documenting specific effects of $1,25(OH)_2D_3$ on macrophages, DCs, and T-cells provides strong evidence of a true physiologic role for this steroid pathway in normal immune function. The ability of some or all of these cell types to produce $1,25(OH)_2D_3$ under some conditions suggests, furthermore, a paracrine model in which appropriate stimuli induce immunomodulatory concentrations of $1,25(OH)_2D_3$ within the microenvironment of an inflamed tissue or lymphoid organ (4, 8, 26, 94). Based on the available data, one would predict that locally stimulated $1,25(OH)_2D_3$ serves a number of functions in the regulation of innate and cognate immune responses as well in the cross talk between the two:

1) *Enhancement of Innate Immunity Through Increased Macrophage Recruitment and Differentiation*: As previously described, early studies demonstrated macrophage-like differentiation of monocytes and myelomonocytic cell lines following exposure to $1,25(OH)_2D_3$ (1, 11, 60, 114). In addition, increased production of macrophage-specific chemokines such as MCP-1, MIP-1α, and MIP-1β (136) and growth factors such as CSF-1 (144) by $1,25(OH)_2D_3$-treated monocytes or bone marrow cells has been recently reported by our group and by that of Zhu et al. These findings implicate $1,25(OH)_2D_3$ in the enhancement of localized innate immune responses and are consistent with an increased susceptibility of vitamin D–deficient animals and patients to infection (10, 51, 126, 139).
2) *Prevention of Autoimmunity Through Inhibition of the Ability of Dendritic Cells to Induce Th-1-type Cellular Immune Responses*: A number of tissue-specific autoimmune diseases are the product of maladaptive Th-1 cellular responses (87, 92). This form of immunity is typically associated with locally destructive lymphocytic infiltration, is stimulated by DCs presenting antigen in the presence of the cytokine IL-12, and is characterized by the production of high levels of interferon-γ (71). Inhibition of factors leading to Th-1 polarization following activation of innate responses is likely to be important for the avoidance of autoimmunity. A number of lines of evidence point to a significant role for $1,25(OH)_2D_3$ in the negative regulation of Th-1–type immunity (76). These include the documented effect of $1,25(OH)_2D_3$ to specifically inhibit DC maturation and production of IL-12 (54, 55, 99, 100, 130) and to directly regulate interferon-γ gene transcription (43, 110, 113). Direct inhibition of IL-2 production and activated T-cell proliferation by $1,25(OH)_2D_3$ likely also contributes to the regulation of Th-1 responses through the limitation of clonal expansion and CD4+ T-cell help to cytotoxic (CD8+) T-cells (12). Furthermore, disease models such as EAE and NOD diabetes that are significantly modified by $1,25(OH)_2D_3$ agonists are classic examples of Th-1 autoimmunity. For both of these models Cantorna et al. and DeLuca & Cantorna have reported that mice deficient in

vitamin D develop clinical disease more rapidly than vitamin D–replete controls (29, 46). Finally, there is evidence from human epidemiological studies that vitamin D status influences the occurrence of Th-1-mediated autoimmunity. Data from the EURODIAB study of risk factors for development of type I diabetes mellitus identified vitamin D supplementation as an independent protective factor (124). A polymorphism of the VDR gene has been reported to be associated with susceptibility to Crohn's disease (119)—a Th-1 mediated form of inflammatory bowel disease. The association between multiple sclerosis and habitation in Northern latitudes has also been postulated to be linked with vitamin D deficiency (28). Taken together these lines of evidence strongly implicate $1,25(OH)_2D_3$ in the negative regulation of Th-1–type cellular immune responses.

3) *Promotion of Self-tolerance Through Permissive Effects on the Generation of Th-2- or T_{reg}-type T-cells*: As described in previous sections, there is evidence from a number of in vitro and animal model studies that administration of $1,25(OH)_2D_3$ agonists is associated with the promotion of Th-2- or T_{reg}-type T-cells (9, 19, 34, 52, 53). It remains to be determined, however, whether these phenomena are the result of preferential suppression of Th-1 responses or represent specific stimulation of alternative T-helper differentiation programs. It is also unclear whether such effects are mediated through modification of DC maturity, through direct effects on T-cells, or, as now seems likely, through a combination of the two. It may be possible to address such questions by further study of animals unresponsive to $1,25(OH)_2D_3$ through knockout of the gene for VDR. Preliminary studies of the immune system of such VDR-deficient mice has demonstrated hypertrophy of subcutaneous lymph nodes with an increased proportion of highly mature DCs (55). These animals have not, however, been found to have widespread autoimmunity or intrinsic defects in in vitro lymphocyte function (86). A better appreciation of the physiological role of $1,25(OH)_2D_3$ in the regulation of immune tolerance and T-helper phenotype may require the generation of in vivo systems in which VDR-deficient immune populations are observed in otherwise normal hosts.

CONCLUSIONS

The studies reviewed here establish important effects of $1,25(OH)_2D_3$ on normal and abnormal immune function. The therapeutic application of these insights is currently in its infancy but the availability of $1,25(OH)_2D_3$ analogs with favorable in vivo profiles has been well documented and it is likely that clinical trials will demonstrate significant beneficial effects for such agents in autoimmune disease and transplantation. As conceptual models of immune system regulation continue to develop it is also likely that important physiological roles for the observed influences of $1,25(OH)_2D_3$ on multiple immune cell types will be identified.

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APPENDIX

Abbreviations: NK cell, natural killer cell; DC, dendritic cell; Th-1, T-helper type 1; Th-2, T-helper type 2; CTL, cytotoxic T lymphocyte; $1,25(OH)_2D_3$, 1α,25-dihydroxycholecalciferol; VDR, vitamin D receptor; TCR, T-cell receptor; DTH, delayed-type hypersensitivity; APC, antigen presenting cell; T_{reg}, regulatory T-cell; SLE, systemic lupus erythematosis; NOD, non-obese diabetic; MBP, myelin basic protein; EAE, experimental allergic encephalomyelitis; DBP, vitamin D–binding protein.

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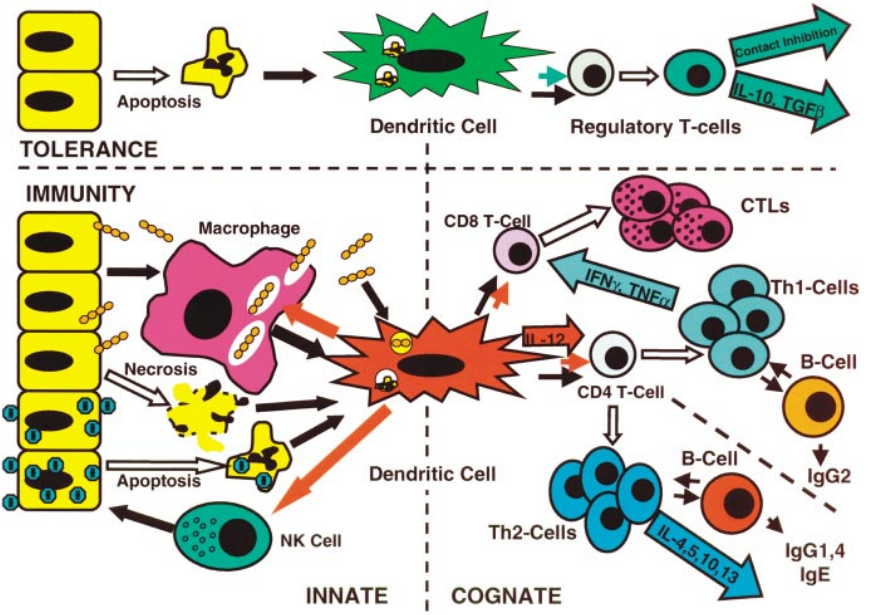

Figure 1 Cross-talk between innate and cognate immunity and its role in determining the nature of antigen-specific immune responses. *Upper panel* (*Tolerance*): Uptake of apoptotic cells by DCs within healthy tissues is associated with trafficking of immature DCs to lymphoid tissues and presentation of self-antigens to regulatory T-cells that act to suppress potentially self-reactive responses by contact inhibition or secretion of immunomodulatory cytokines (IL-10, TGFβ). *Lower panel* (*Immunity*): In the presence of microbial infection the release of proinflammatory factors or the death of infected cells by necrosis or apoptosis results in (*a*) recruitment of macrophages and NK cells, (*b*) amplification of innate responses by macrophage-derived products and NK-mediated killing of infected cells, (*c*) antigen uptake by resident and recruited DCs with concomitant DC maturation through multiple innate signals, (*d*) additional amplification of innate responses by DC-secreted products, (*e*) trafficking of mature DCs to lymphoid tissues, and (*f*) DC interactions with CD4+ T-cells to induce Th-1 or Th-2 type cognate immune responses. Th-1 responses are initiated under the influence of IL-12 produced by mature DCs and are characterized by secretion of IFNγ and TNFα; provision of help for the activation of CD8+ into antigen-specific cytotoxic T lymphocytes (CTLs), and interactions with B-cells to promote the generation of antibodies of the IgG2 isotype. Th-2 responses are generated by mature DCs in the absence of IL-12 production and are characterized by secretion of IL-4, IL-5, IL-13, and IL-10 and by interactions with B-cells to promote the generation of antibodies of the IgE, IgG1, and IgG4 isotypes.