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REVIEW ARTICLE

Vitamin D and ageing

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Abstract Within the past three to four decades a revolution has occurred in our understanding of vitamin D and its effects. Sundry laboratory and epidemiologic studies have revealed that the active metabolite of vitamin D controls and/or ameliorates various pathologies. As presented here, there is substantive evidence that vitamin D may play a positive and important role in the ageing process. This evidence arises from detailed consideration of various biological mechanisms and processes by which vitamin D operates as well as specific examples of its exerting control/amelioration of various human maladies which contribute to ageing. Arguments are advanced that vitamin D appears to play a major positive role in biogerontology by reducing susceptibility in the elderly to chronic degenerative diseases. It is strongly recommended that the positive role of vitamin D in ageing be taken into account by gerontologists and biogerontology researchers.

Keywords Vitamin D · Ageing · Immunology · Infection · Hormesis · DNA damage control

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Introduction

Our understanding and appreciation of how vitamin D mediates biological responses have entered a new era. Historically, most interest in vitamin D had been relegated to its actions in calcium homeostasis and in bone formation. However, over the past few decades new evidence has emerged from laboratory and epidemiologic studies showing many additional physiological systems in which vitamin D generates biological responses. These include, amongst others, the immune, heart-cardiovascular, muscle, pancreas, and brain systems; as well as involvement in control of the cell cycle and thus of the disease process of cancer (Norman 2008). In addition, reasons have been advanced which strongly suggest that vitamin D provides protection against low-level radiation damage (Hayes 2008a). Many of the recently uncovered vitamin D-responding physiological systems are intimately connected with ageing.

Succeeding sections of this review will be devoted to discussion of topics intimately related to vitamin D-induced biogerontology effects, particularly vitamin D's role in combating/ameliorating age-associated pathologies. These include consideration of the general biological processes undergirding vitamin D's pleiotropic activities, including its photochemistry, biochemistry, metabolism, functions and hormesis, as well as its sufficiency in the elderly and supplementation. Specific biological mechanisms are discussed, including control of DNA damage, cell cycles, cell

proliferation, cell differentiation, cellular communication, antiangiogenesis, antioxidation, programmed cell death, and immunology. Vitamin D's protections against various age-related maladies are discussed, including protecting against autoimmunity, cardiovascular and neurological diseases, and macular degeneration. Concerns which have been expressed about vitamin D's effectiveness will also be considered.

Vitamin D: photochemistry, biochemistry, metabolism and functions

There are several forms of vitamin D, two of which are of major importance: vitamin D_3 being of primary importance and vitamin D_2 less so. Vitamin D_3 (cholecalciferol) is found in a limited number of natural food sources, but more importantly is produced in the skin by sunlight. Solar ultraviolet B (UV-B) photons penetrating the skin cause the robust photolysis of provitamin D_3 (the lanolin cholesterol derivative 7-dehydrocholesterol) to previtamin D_3. Once formed, previtamin D_3 undergoes rapid thermally-induced transformation to more thermodynamically stable vitamin D_3 (cholecalciferol) which then exits the skin and is transported in the circulation bound to plasma proteins. In North America and Europe dietary vitamin D_3 intake is dwarfed by solar-induced D_3 (DeLuca 2004). Vitamin D_2 (ergocalciferol) is found in some plant foods and is manufactured through ultraviolet irradiation of yeast and the plant sterol precursor, ergosterol (Yetley 2008). Circa 2006, it was reported that the major vitamin D medical prescription in North America is for vitamin D_2, not vitamin D_3; and that vitamin D_2 should not be regarded as a nutrient suitable for supplementation or fortification (Houghton and Vieth 2006). Physicians utilizing vitamin D_2 should be aware of its markedly lower potency and shorter duration of action relative to vitamin D_3 (Armas et al. 2004). There are also vitamin D molecules in which chemical modifications have been made to obtain synthetic analogues. Circa 2008, more than 2,000 vitamin D analogues have been synthesized (Norman 2008).

Vitamin D itself is metabolically inactive and must be metabolized by sequentially being given hydroxyls in a tightly regulated multistep process (DeLuca 1997). The first of these hydroxylations, which takes place primarily in the liver by the enzyme 25-hydroxylase, is the enzymatic conversion of vitamin D (either cholecalciferol or ergocalciferol) to the inactive hormonal precursor 25-hydroxyvitamin D, 25(OH)D, which is the predominant circulating form of vitamin D in the blood. The second of these hydroxylations occurs mainly but not exclusively in the proximal convoluted tubule cells of the kidney by the enzyme 25(OH) vitamin D 1α-hydroxylase; starting with a glomerular filtration of 25(OH)D bound to vitamin D binding protein followed by megalin receptor mediated tubular cell reabsorption before it is 1-alpha-hydroxylated. Many extrarenal tissues, including skin, brain, breast, prostate, colon, vascular smooth muscle, macrophages, endothelium, etc., also have the 1α-hydroxylase enzyme to convert 25(OH)D to the pluripotent secosteroid hormone $1,25(OH)_2D$ (although to a lesser extent than the kidney); and to use it to affect numerous cells and tissues, possibly by the same process as in the kidney (Lips 2006). Circulating vitamin D and 25(OH)D may be deposited in adipose and muscle tissue with obesity-associated fat tissue sequestration (Davis 2008). While some 37 other vitamin D_3 metabolites have been isolated and characterized (Bouillon et al. 1995), the only known and conclusively proven hormonal form of vitamin D is $1,25(OH)_2D_3$, also known as calcitriol (DeLuca and Zierold 1998). Calcitriol is a full member of the endocrine system and as such interacts with virtually every organ of the body and regulates a variety of genes or gene products in different genetic circuits (Minghetti and Norman 1988). Henceforth in this review the nomenclatures "the hormonally active form of vitamin D," "the active metabolite of vitamin D," as well as "calcitriol" are taken to be synonymous with $1,25(OH)_2D_3$ (while "vitamin D" may refer to either vitamin D_3 or D_2).

It is generally accepted that the main long-term actions of the biologically active form of vitamin D are genomic effects mediated via the genomic pathway involving binding of the hormone to specific high-affinity intracellular vitamin D receptors (VDRs) present in essentially all tissues and cells in the body (DeLuca 2004). $1,25(OH)_2D_3$ initiates the physiological responses of $\geq$36 cell types that possess VDRs, with the number of detected target organs having increased $\approx$9-fold since the early 1970s (Norman 2008). Vitamin D and its analogues can easily pass through biological membranes and bind

with high affinity to the nuclear receptor VDR. In turn, the receptor-hormone complex binds to a region of the DNA strand known as the promoter-specific vitamin D response element (VDRE), usually as a heterodimer with a required protein partner for DNA binding, the steroid protein receptor retinoid X receptor (RXR), within the promoter region of vitamin D response genes (VDRGs). Promoter-specific binding of the complex results in altered expression of the attendant gene (Omdahl and May 1997). Circa 2005 it was known that calcitriol activates VDRs to transcribe (or repress) 913 target genes (Wang et al. 2005), and might possibly affect the expression of many more (Marshall 2008). The net effect of the VDR (and more importantly the parathyroid hormone PTH) on its classical target tissues (intestine, kidney, and bone) is to regulate plasma calcium ion Ca^{2+} levels to achieve/maintain Ca^{2+} homeostasis [as will later be discussed, vitamin D induces an influx of $Ca^{2+}$from the intracellular pool ($[Ca^{2+}]_i$) that provides critical signals in programmed cell death and growth inhibition (Mathiasen et al. 2002)]. Interestingly, many cancer cells possessing VDRs have retained the ability to respond to the growth regulating effects of $1,25(OH)_2D_3$ (Holick 1995), leading to the recognition of VDRs being involved in cellular proliferation, differentiation, angiogenesis, immunomodulation, and control of other hormonal systems (Holick 2007). It has also led to the recognition that $1,25(OH)_2D_3$ inhibits the proliferation and induces maturation of both normal and tumor cells that posses a VDR (Nagpal et al. 2005).

In addition to its genomic effects, $1,25(OH)_2D_3$ and its analogues can also elicit biological responses that are too rapid (seconds to 1–2 min) to involve changes in gene expression and appear to be mediated by cell membrane VDRs resulting in second messenger signaling or phosphorylation of intracellular proteins (Dawson-Hughes 2008). Rapid nongenomic actions of $1,25(OH)_2D_3$ have been observed both at the cellular level, e.g., calcium transport across a tissue, and at the subcellular level, e.g., membrane calcium transport and changes in intracellular second messengers (Bouillon et al. 1995). These rapid nongenotropic effects include activation of protein kinases and increases in intracellular calcium $[Ca^{2+}]_i$ levels (Dusso et al. 2005). Binding of $1,25(OH)_2D_3$ to a membrane receptor can result in the activation of numerous signaling cascades resulting in the rapid opening of voltage-gated Ca^{2+} channels and increase in $[Ca^{2+}]_i$ levels, which may subsequentially mediate proliferate effects (Deeb et al. 2007). The demonstrated antiproliferative, prodifferentiative and immunomodulatory activities induced by $1,25(OH)_2D_3$ and its analogues may be mediated by both the genomic and nongenomic mechanisms (Holick 2006). Those of a biochemical bent are directed to more detailed discussions of these topics in Feldman et al. (1997) and Holick (1999).

Vitamin D: hormesis

Hormesis is the biological and toxicological concept that small quantities have opposite effects from large quantities. In the hormesis paradigm agents induce dose–response relationships having two distinct phases (i.e., biphasic, non-monotonic) with biologically opposite effects at different doses (Hayes 2008b). Most commonly there is a stimulatory or beneficial effect at low doses and an inhibitory or toxic effect at high doses. Evidence for nutritional hormesis has been presented for essential vitamin and mineral nutrients, dietary energy restriction, alcohol, natural dietary and some synthetic pesticides, some herbicides, and acrylamide by Hayes (2007). There are laboratory studies attesting to vitamin D-induced biphasic dose-responses (Aubin and Heersche 1997). Cited by Stumpf (2006) as exemplifying hormesis in humans is the fact that low doses of vitamin D have stimulatory effects promoting epidermal wound healing in contrast to high doses inhibiting psoriasis. A longitudinal nested case–control study of prostate cancer risk showed intriguing biphasic U-shaped vitamin D dose–response (Tuohimaa et al. 2004).

Vitamin D: sufficiency in the aged and supplementation

Measurement of serum concentration of 25-hydroxyvitamin D [25(OH)D], the major circulating metabolite of vitamin D, is regarded as the best clinical indicator of vitamin D status in humans. It is generally agreed that optimum 25(OH)D concentrations in humans should be defined as $\geq$75–80 nmol/l (Smith et al. 2009), with concentrations <25 nmol/l considered severely deficient and 25–50 nmol/l considered insufficient (Lips 2007). Circulating 25(OH) D levels

in early humans were surely higher than what is now regarded as normal (Vieth 1999). Natural 25(OH)D levels found in humans who live or work in a sun-rich environment are >100 nmol/l (Cannell et al. 2008), while vitamin D toxicity arising from sunlight overexposure has never been reported (Cantorna and Mahon 2004).

Vitamin D inadequacy constitutes a largely unrecognized epidemic in many populations worldwide, being reported in healthy children, young adults (especially African-Americans), middle-aged adults, as well as in the elderly (Prentice 2008). Disturbingly, a significant temporal reduction in the vitamin D status of the United States population has been reported in surveys conducted over years 1988–1994 vis-à-vis 2000–2005 (Looker et al. 2008). There is strong evidence that 25(OH)D is significantly lower in the elderly compared to a younger control population (Utiger 1998). There is also evidence that the obese have lower 25(OH) D and $1,25(OH)_2D$ and higher PTH levels (Blum et al. 2008). In populations absent of disease and consuming a normal Western diet, vitamin D metabolism begins to change around midlife. Dietary calcium and vitamin D as well as cutaneous-produced vitamin D may decline, serum growth hormone (GH) and insulin-like growth factor type I (IGF-I) begin to decrease, and renal function begins to deteriorate. As consequence of these changes 25(OH)D decreases, calcium absorption in the intestine diminishes (diet calcium and VDR concentration decrease), and calcium bioavailability to meet serum demands declines. This stimulates PTH secretion, but because of declining renal function and loss of the normal trophic effects of testosterone, estrogen, Fibroblast Growth Factor 23 (FGF23), and GH/IGF-I on 1α-hydroxylase activity, production of $1,25(OH)_2D$ either decreases, remains unchanged, or increases modestly depending on the individual (Halloran and Portale 1997).

Since casual everyday skin exposure to sunlight provides most humans with their vitamin D requirements, any skin changes are of utmost importance. Ageing has a dramatic effect on the skin with vitamin D_3 production decreasing because the skin becomes thinner, decreasing linearly with age in humans after age 20 years (Tan et al. 1982). By the age of 70 years the concentration of provitamin D_3 in the epidermis, where most previtamin D_3 synthesis occurs, can be decreased by as much as 75% (MacLaughlin and Holick 1985). Nevertheless, there still remains reservoirs of cutaneous provitamin D_3 in the elderly that with sufficient sunlight exposure permits the production of increased and adequate concentrations of vitamin D_3 (Chuck et al. 2001). This fact is consistent with results of the "Third National Health and Nutrition Examination Survey" which revealed that persons aged 60 or more years who participated in daily outdoor activities had mean 25(OH)D concentrations similar to those aged 20–39 years, suggesting that the elderly with adequate solar exposure can synthesize enough vitamin D_3 from outdoor activities to maintain levels similar to those of young adults (Scragg and Camargo 2008).

A question that naturally arises is whether vitamin D supplementation produces salutary effects in the elderly? It should be noted that the elderly at high risk of vitamin D inadequacy often do not follow regular daily dosing requirements. For example, despite receiving counseling on the importance of vitamin D and calcium supplementation, 76% of elderly patients with hip fractures did not comply with recommendations (Segal et al. 2004). This is not surprising given that compliance declines as the number of medications increases, and the elderly often take many medications. Many of the studies of the effects of supplementation on the elderly are centered about physical performance. After adjustments for age, gender, chronic diseases, degree of urbanization, body mass index, and alcohol consumption; vitamin D status (as measured by 25(OH)D levels) was found to predict physical performance in older persons as well as its decline over time (Wicherts et al. 2007). Available evidence suggests that the elderly need a mean serum 25(OH)D concentration of $\geq$65 nmol/l to improve muscle performance and reduce the risk of falling and $\geq$75 nmol/l to reduce the risk of fracture (Dawson-Hughes 2008). Since many of the elderly have serum 25(OH)D concentrations below these levels, it was further reported that supplementation is likely to provide significant benefit to the elderly segment of the population.

Data from large prospective epidemiological studies suggest that vitamin D supplementation reduces the incidence of Type I diabetes, rheumatoid arthritis, and multiple sclerosis (Cantorna and Mahon 2004). Importantly, a meta-analysis of eighteen randomized trials demonstrated a significant reduction in all-

cause mortality among older individuals assigned vitamin D supplementation (Autier and Gandini 2007). The authors of this study stated that population-based, placebo-controlled randomized interventional trials with total mortality as the main end point should be organized for confirmation of their findings. As will be detailed later, a study meeting many of these requirements has reported that interventional vitamin D supplementation in postmenopausal women reduced cancer risk (Lappe et al. 2007).

Vitamin D: control of DNA damage, cell cycle and cell proliferation

DNA is usually regarded as the most critical cellular target for production of carcinogenic and mutagenic effects arising from both endogenous and such exogenous processes as radiation, drugs, and environmental chemicals. Endogenous and exogenous processes can result in various directly and indirectly induced DNA lesions with double-strand chromosomal breaks (DSBs) commonly accepted as the mechanistic surrogate for carcinogenesis and a major risk for cancer (Steel 2002). $1,25(OH)_2D_3$ receptors preferentially bind to double-stranded DNA rather than to single-stranded DNA or RNA (Pike 1985). For rodent and cell culture models it has been reported that vitamin D_3 at a concentration range of 20–50 nmol/l prevents endogenously- and exogenously-induced double-strand breaks and DNA-carcinogen adducts as well as stabilizing chromosomal structure (Chatterjee 2001).

Calcitriol upregulates proteins that control the cell cycle and decreases cell proliferation of both normal and aberrant cells (Holick 2007). Treatment of cell types with vitamin D_3 and its analogues has been found to cause an arrest of cell cycle progression in the G_1-phase resulting in decreased number of cells in the S-phase complemented by accumulation of cells in G_0–G_1 phase. The $1,25(OH)_2D_3$-VDR system induces growth arrest in both cancer and noncancerous hyperproliferative disorders by targeting several key proteins regulating the G_1–S phase transition, such as cyclins, CDKs cyclin-dependent kinases (a family of nuclear protein kinases), and CDKIs cyclin-dependent kinase inhibitors (Dusso et al. 2005). Calcitriol also upregulates the genes which control the p53 as well as other tumor suppressor proteins which contribute to cell cycle arrest as well as apoptosis and DNA repair after genotoxic or non-genotoxic stresses (Ohnishi et al. 2002). These vitamin D-induced molecular events have earned the sobriquet "guardians of the cell cycle" (Lamprecht and Lipkin 2003).

Vitamin D: cell differentiation

Ageing is associated with a deterioration of cell differentiation and protein synthesis capacity in most tissues, particularly in the post-mitotic tissues (Ji 2008). One of the universal characteristics of a cancer cell is that it appears to be "immortalized" and partially but not terminally differentiated. $1,25(OH)_2D_3$ and its analogues are potent prodifferentiation mediators of a large number of normal and malignant cells (Holick 1995). Pulsatile release of ionized calcium from intracellular stores, $[Ca^{2+}]_i$, including the endoplasmic reticulum, induces terminal differentiation with $1,25(OH)_2D_3$ enhancing this release (Mathiasen et al. 2002). $1,25(OH)_2D_3$ concomitantly upregulates or stimulates the expression of differentiation-associated genes while it controls the replication-linked cell cycle genes (Minghetti and Norman 1988). Recent microarray studies of gene expression profiles in cancer cells have highlighted the capacity of the active metabolite of vitamin D and its analogues to drive malignant cells to a more differentiated state (Masuda and Jones 2006). VDR knockout studies confirm the role of vitamin D in differentiating target organs (Ylikomi et al. 2002). The effects of $1,25(OH)_2D_3$ on growth inhibition and differential induction in a number of clinical applications have been discussed by Walters (1992), including its therapeutic potential in combating various malignancies and treating psoriasis. Rapidly proliferating and poorly differentiated cultured human epidermal keratinocytes can be induced to terminally differentiate by $1,25(OH)_2D_3$ (van den Bemd et al. 2000).

Vitamin D: cellular communication

Various studies have shown that decreased or lost intercellular communication is strongly associated with aberrant cell growth diseases, including cancer (Yamasaki 1990). Contrarily, enhanced intercellular

communication inhibits aberrant proliferation, so that upregulation of intercellular communication would be expected to prove beneficial.

Vitamin D and its metabolites have been found to enhance intercellular communication among adjacent cells through gap junction intercellular communication (GJIC) with the connexin (cx) proteins that constitute junctional systems declining when vitamin D concentration is low (Fernandez-Garcia et al. 2005). Tight-junction protein claudins which form paracellular channels for Ca^{2+} ions between neighboring cells have been found to be upregulated in intestinal absorptive cells in vitro and in vivo by $1,25(OH)_2D_3$ through its vitamin D receptors (Fujita et al. 2008). By enhancing intercellular communication vitamin D appears to inhibit malignant cell transformation and facilitates the passage of regulatory substances between carcinogen initiated and normal cells (Banerjee and Chatterjee 2003). Calcitriol induces GJIC in human skin fibroblasts at relatively low concentration with concomitant increases in VDR-dependent cx43 protein and cx43 mRNA levels, whereas human skin fibroblasts devoid of functional VDR showed no such effects. This reliance on nuclear receptors suggests that calcitriol alters the expression of endogenous genes in treated cells and affects GJIC at the level of transcription or of mRNA stability via the VDR (Clairmont et al. 1996). Observations suggest that vitamin D_3 may prevent human renal cell carcinoma by preserving GJIC during carcinogenesis (Fujioka et al. 2000). It has been stated that promotion of cellular communication by vitamin D-induced enhancement of Ca^{2+} and other messenger signals should play a positive and constructive role in combating age-related pathologies (Yamasaki and Naus 1996).

Vitamin D: antiangiogenesis

Angiogenesis is the physiological process involving the formation of new blood vessels from a preexisting vascular bed. It is a normal process in growth, development, inflammation and wound healing, as well as being of fundamental importance in several pathological states and transition of tumors from dormant to malignant states (Folkman 1995). Modulation of angiogenesis is a strategy for the treatment of pathologies categorized by their reliance on a vascular supply (Mantell et al. 2000). If a cell becomes malignant vitamin D_3 can prevent angiogenesis by inducing apoptosis and thereby reducing the potential for malignant cell survival (Holick 2007). The antiangiogenic role of vitamin D_3 may be an important factor in combating and controlling pathologies associated with the ageing process.

Both in vivo and in vitro findings indicate that $1,25(OH)_2D_3$ and its analogues inhibit both the proliferation of some tumor derived endothelial cells (TDECs) and expression of vascular endothelial growth factor (VEGF) proteins that induce tumor angiogenesis (Bernardi et al. 2002). Vitamin D_3 is effective in controlling macular degeneration due to its efficacy in combating VEGF-induced proliferation of capillaries into the retina causing loss of vision. Its efficacy as an antiangiogenic and antiinflammatory agent has been suggested to explain the protective association between vitamin D and age-related macular degeneration reported in the "Third National Health and Nutrition Examination Survey" (Parekh et al. 2007). In addition to controlling macular degeneration, there are also a whole host of other age-associated pathologies where antiangiogenesis therapy would be expected to prove effective. As proposed by Pepper (1997), these include ocular neovascularization, hemangioma, rheumatoid arthritis, and atherosclerotic plaque neovascularization. The antiangiogenic actions of vitamin D_3 should play a positive and productive role in combating these and sundry other age-related pathologies.

Vitamin D: antioxidation

During the course of normal metabolism, reactive oxygen species (ROS) are produced from within the respiratory chain of mitochondria with the ability to oxidize and damage a variety of cellular constituents. It has been proposed that age-associated accumulation of mitochondrial deficits is likely to be a major contributor to cellular-, tissue-, and organismal-ageing (Shigenga et al. 1994), being termed the free radical theory of ageing (Harman 2006). Free radicals are normally neutralized by efficient antioxidant enzymes. A large number of antioxidative agents have been shown to exhibit protective effects in cell

culture and animal models relevant to age-associated disorders (Ofodile 2006).

It should be noted that human antioxidant supplementation has pros and cons for any population that raise numerous questions, issues, and challenges (Seifried et al. 2003). It is important to note that prooxidants may also have an important role in preventing and combating ageing with low-ROS concentrations serving vital physiological signaling and cell membrane functions for healthy ageing (Linnane et al. 2007). ROS-induced protection mechanisms include apoptosis, which deletes precancerous cancer, virus-infected and other cells threatening human health and which will be discussed in detail later; phagocytosis, which fights infectious microorganisms; as well as sundry detoxification reactions (Salganik 2001). It has also been proposed that low-level ROS induces hormetic effects, and that it is only at high levels that ROS inflicts cell damage causing underlying disease processes (Abete et al. 2008).

The antioxidative role of vitamin D_3 acting as a free radical scavenger had earlier been discussed by Willson (1992). There have been many later laboratory reports of vitamin D_3 exerting protective antioxidative actions, some of which have been discussed by Chatterjee (2001). An in vitro study reported that vitamin D_3 not only suppressed autooxidation but may also be one of the most powerful antioxidants in biological organisms based on the fact that it was some 10^3 more potent than a water soluble vitamin E analogue in inhibiting zinc-induced CNS oxidative stress (Lin et al. 2005). Interestingly, $1,25(OH)_2D_3$ has been reported to protect some nonmalignant human prostate epithelial cell lines, but not some malignant human prostate epithelial cells from oxidative stress-induced cell death (Bao et al. 2008). In addition, it should be noted that vitamin D may equally well serve as a hormetic agent triggered by low-level ROS. The vitamin D-mediated hormetic agency could be any of those discussed in this review.

Vitamin D: programmed cell death (apoptosis and autophagy)

Programmed cell death (PCD) may confer advantages during an organism's life cycle and play an important role in ageing. There are at least two types of PCD: apoptosis and autophagy. While both types of PCD involve synthesis of distinct proteins, they should not be considered mutually exclusive phenomena; rather they appear to reflect a high degree of flexibility in a cell's response to changes of environmental conditions, both physiological and pathological (Bursch et al. 2000). The immediate following subsections of this review will separately discuss apoptosis and autophagy.

Programmed cell death: apoptosis

Apoptosis is defined by a variety of distinct morphological and biochemical changes which can be mediated by the caspases family of proteins, which are expressed as inactive zymogens and are proteolytically processed to an active state following an apoptotic stimulus (Johnstone et al. 2002). The initial common view was that apoptosis would primarily have a negative impact on ageing (Lockshin and Zakeri 1990). Current thinking is more on the lines of the propositions that apoptosis is an important defense mechanism in maintaining genetic stability with centenarians having "aged successfully" because their cells are more prone to apoptosis (Franceschi et al. 1992), and that ageing retardation could be due to apoptosis upregulation (Warner et al. 1997). Apoptosis enhances the elimination of various damaged and dysfunctional cells presumably caused by oxidative stress, glycation and DNA damage, and thereby potentially plays an important role in the ageing process (Higami and Shimokawa 2000).

Vitamin D_3 and its derivatives have been shown to influence the regulation of genes and protein products thought to promote active apoptotic cell death in numerous (but not all) normal and cancer cell types (evidence for the role of $1,25(OH)_2D_3$-induced apoptosis in antiangiogenesis and antioxidation has already been discussed in this review). Consideration will now be given to some of the mechanisms undergirding vitamin D_3 and apoptosis. Oxidants and antioxidants determine cell fate, including the modulation of cell death (Chandra et al. 2000); and as already noted in the discussion on antioxidants, reactive oxygen species may be essential biochemical intermediates in the progress of many forms of apoptosis (Slater et al. 1995), with apoptosis being

triggered or blocked dependent on the severity of oxidative stress (Hampton and Orrenius 1998). The p53 apoptotic regulator gene is a direct transcriptional activator of the human bax gene (Miyashita and Reed 1995). Vitamin D_3 and its derivatives have been shown to upregulate wild type p53 protein expression in concert with decreased expression of the antiapoptotic bcl-2 proteins, bcl-2 and bcl-X_L (Danielsson et al. 1997). Since p53 inhibits replication, its loss or reduction by insufficient vitamin D metabolites cuts the doubling time of the cell thereby conferring selective reproductive advantage on the progeny. Other suggested mechanisms for the apoptotic effect of vitamin D_3 include down-regulation of the antiapoptotic IGF receptor, activation of the sphingomyelin-ceramide-ganglioside GD3 signaling pathway, and reduced expression of Akt, a kinase that regulates cell survival signals (Masuda and Jones 2006).

Another factor to be considered in the apoptotic actions of vitamin D is the possibility that it induces an efflux of Ca^{2+} from the intracellular pool ($[Ca^{2+}]_i$) that provides a critical signal in programmed cell death and growth inhibition (Chatterjee 2001). It has been explicitly declared that a supramicromolar elevation of intracellular free calcium ($[Ca^{2+}]_i$) is consistently required to induce the execution phase of apoptosis (Tombal et al. 2002). $1,25(OH)_2D_3$-induced apoptosis is associated with a sustained increase in concentration of $[Ca^{2+}]_i$ resulting from depletion of the endoplasmic reticulum Ca^{2+} stores via voltage-insensitive Ca^{2+} channels (Sergeev 2005). Vitamin D_3-induced intracellular calcium increase has been proposed as activating calcium-dependent pro-apoptotic proteases μ-calpain and capase 12, with calpain acting as the major execution protease in apoptosis-like death (Mathiasen et al. 2002).

Programmed cell death: autophagy

Sometimes referred to as "a garbage disposal mechanism" (Mathew and White 2007), autophagy's purpose is to dispose of defective organelles and macromolecular structures, as well as cytosolic components such as damaged and aggregate-prone proteins. Autophagy is a catabolic process involving the degradation of a cell's own components through the endosomal-lysomal system in which cytoplasmic constituents of cells are engulfed within a cytoplasmic vacuole and delivered to the lysosome for degradation (a process of "self-consumption"), and is associated with formation of autophagosomes and depends on autophagy proteins (Tsujimoto and Shimizu 2005).

Autophagic cell death of human SCC25 head and neck squamous carcinoma cells has been found to be remarkably stimulated by $1,25(OH)_2D_3$ treatment, but only in cells deficient in expression of the cyclin-dependent kinase inhibitors $p19^{INK4D}$ and $p27^{KIP1}$. In contrast, vitamin D_3 did not induce autophagy in U937 leukemia cells with knockdown of $p19^{INK4D}$, suggesting that this effect of $p19^{INK4D}$ deficiency on autophagy induction is cancer cell specific (Tavera-Mendoza et al. 2006). A study showed that a vitamin D analogue induced massive autophagic cell death via a pathway involving beclin-1 acting as an autophagy-inducing tumor suppressor gene and playing an important role in human tumor suppression (Yue et al. 2003). Autophagic cell death has been related to free cytosolic calcium ($[Ca^{2+}]_c$) induced by vitamin D compounds in a beclin-2 regulated fashion (Hoyer-Hansen and Jaattela 2007). The earlier report by Mathiasen et al. (2002) that vitamin D compounds induced autophagy (and apoptosis) in MCF-7 cancer cells has been explained by the nature of the calcium ion itself (Swerdlow and Distelhorst 2007). Current studies to define the mechanism(s) by which vitamin D_3 and its analogues respond to ionizing radiation in breast tumor cells suggest that these effects are mediated in large part through the promotion of autophagic cell death, with the residual surviving cell population remaining in a senescent growth arrested state with minimal recovery for proliferative capacity (Gewirtz 2007).

Studies indicate a role for the induction of autophagy in host defenses against mycobacterial infection. Autophagy can combat infections by presenting foreign antigens for recognition by the immune system and by killing bacteria ingested by cells. In human macrophages, vitamin D-mediated induction of antimicrobial peptides (AMPs) appears to be an important player in combating *M. tuberculosis* and *M. smegmatis* (Liu and Modlin 2008). As already noted, macrophages are important vitamin D targets since they are a potential source of the 1α-hydroxylase enzyme that converts $25(OH)D_3$ to VDR binding $1,25(OH)_2D_3$ (Froicu and Cantorna 2007).

Vitamin D: immunology

With age there is a progressive decline in immune response with peculiar susceptibility to infectious diseases, autoimmunity and cancer as well the pathogenesis of important age-related diseases such as cardiovascular, neurodegenerative, diabetes and osteoporosis (Capri et al. 2006). Until about 1980, no one had imagined that vitamin D might play a role in the functioning of the immune system (DeLuca and Cantorna 2001). Since then it has become appreciated that the hormonally active form of vitamin D has intriguing immunomodulatory/immunoregulatory/autoimmune properties—suppressing immune responses under some conditions and enhancing them in others (Nagpal et al. 2005). These properties arise from calcitriol's ability to influence gene expression in immune system cells and cytokine immune system signaling expression by other cells (Mathieu and Adorini 2002).

Many cells involved in immune response (e.g., antigen-presenting cells, thymocytes, different subtypes of T cells, including T helper Th1, Th2 and T suppressor cells, and natural killer cells) express VDRs either in the resting state or after cell activation, and are sensitive to the presence of $1,25(OH)_2D_3$ (Bouillon et al. 1995). Effects of vitamin D_3 on the immune system are manifold and include regulation of T cell proliferation and function, suppression of T cell activation, induction of regulatory T cells, affecting cytokine secretion patterns and antigen-presenting cells (APCs), in particular dendritic cells (DCs), and inducing both pro- and anti-apoptotic activity (May et al. 2004). With regard to DCs, $1,25(OH)_2D_3$ and its analogues have been found to inhibit their differentiation and maturation, to promote their spontaneous apoptosis, and to inhibit DC-dependent T cell activation (Penna and Adorini 2000). Moreover, 1-α-hydroxylase, the enzyme responsible for the final and rate limiting hydroxylation step in the synthesis of $1,25(OH)_2D_3$, may be upregulated and able to synthesize and secrete $1,25(OH)_2D_3$ in a regulated fashion in APCs (Mathieu and Adorini 2002). This process might be a negative feedback loop in inflammation, with a defect in this system being an additional element in tipping the balance towards autoimmunity.

The widespread presence of VDRs in different immune systems, the regulated expression of 1α-hydroxylase by specific immune signals, and DCs regulation by immune signals suggest a paracrine immunomodulatory role for the $1,25(OH)_2D_3$ system down-regulating immune response locally in sites of inflammation (Overbergh et al. 2000). Strikingly, $1,25(OH)_2D_3$ uses several different molecular mechanisms to regulate cytokine expression, either directly targeting transcription initiation and regulation, or indirectly interfering with other signaling pathways. Moreover, APCs as well as T cells can be direct targets of the immunomodulatory effects of $1,25(OH)_2D_3$, leading to the inhibition of pathogenic effector T cells and enhancing the frequency of T cells with regulatory properties, largely via induction of immunological tolerant (tolerogenic) DCs resulting in T cell anergy, i.e., the antigen-specific T cells remain present but fail to function fully (van Etten and Mathieu 2005).

Vitamin D_3 enhances the capacity of the innate immune system to produce endogenous antibiotics. It plays an immunomodulatory role in infection risk by regulating the synthesis and actions of naturally occurring defensin molecules against bacterial antigens (Adams et al. 2007; Defensin is a generic name reserved for an endogenously synthesized antimicrobial agent). Macrophages, monocytes, neutrophils, natural killer cells, and epithelial cells increase expression of antimicrobial proteins on exposure to microbes, an expression that can be stimulated by vitamin D_3 (Griffin et al. 2000). As already noted, there is evidence that vitamin D-mediated induction of antimicrobial peptides (AMPs) combats *M. tuberculosis* and *M. smegmatis* via a process enhanced by autophagy. Vitamin D's stimulation of AMPs in epithelial cells lining the respiratory tract plays a major role in protecting the lung from infection (Cannell et al. 2006). Pathogenic microbes stimulate the production of the hydroxylase which converts $25(OH)D_3$ to $1,25(OH)D_3$ and which in turn activates a suite of genes involved in defense.

Vitamin D: autoimmunity

Autoimmune diseases are characterized by the targeted destruction of self-tissue by the immune system. T cells are characterized by their ability to discriminate among antigens, and are what normally prevents autoimmunity. For reasons that are not yet

fully understood, people and animals with autoimmunity disease have many T cells that recognize self-tissues. Various human and laboratory evidence indicates that vitamin D plays a role in the etiology of autoimmunity by serving in the development of self-tolerance. While vitamin D deficiency clearly affects the immune system, especially T cell-mediated immunity, vitamin D in excess actually suppresses certain aspects of the immune system. This has led to the use of vitamin D compounds to suppress certain autoimmune disorders (DeLuca 2004). The presence of vitamin D receptors (VDRs) in both the thymus and peripheral T cells suggested a role for vitamin D in the development and function of T cells, and the reasonable hypothesis that vitamin D and $1,25(OH)_2D_3$ are selective regulators of the immune system. Normal T cell function and the prevention of autoimmune disease require signaling via $1,25(OH)_2D_3$ and the VDR. In the absence of vitamin D and signals delivered through the vitamin D receptor, auto-reactive T cells develop; while in the presence of $1,25(OH)_2D_3$ and a functional vitamin D receptor, the balance in the T cell response is restored and autoimmunity avoided (Cantorna 2006). CD4+ T cells control experimental autoimmunity with experimental data suggesting that vitamin D directly or indirectly regulates their differentiation and activity to suppress autoimmune pathology. The net result is a decrease in the Th1-driven autoimmune response and decreased severity of symptoms (Cantorna et al. 2004).

Apoptosis plays an important role in defense against autoimmune disease during ageing (Warner 1999). The important role of vitamin D in promoting apoptosis in the ageing process has already been discussed in this review. The Fas antigen is a member of the superfamily of complementary receptors and ligands that has been identified as a key cell surface receptor involved in apoptotic cell death. A Fas gene homolog has been identified in humans with mutations in this gene leading to abnormal lymphoproliferation and autoimmunity, indicating a crucial regulatory role of Fas and thereby providing a molecular basis for some human autoimmune diseases (Rieux-Laucat et al. 1995). Vitamin D's well-established general role in promoting apoptosis would appear to manifest a specific role in promoting Fas-induced apoptosis.

There is substantial evidence attesting that vitamin D plays an important and positive role in combating human autoimmunity disorders. As already noted, data from large prospective epidemiological studies suggest that vitamin D supplementation reduces the incidence of such autoimmunity diseases as Type I diabetes, rheumatoid arthritis, and multiple sclerosis (Cantorna and Mahon 2004). Patients with these diseases as well as inflammatory bowel disease express different VDR polymorphisms associated with their disease, have low circulating levels of $25(OH)D_3$, and are at increased risk for bone fractures. In infants, 2,000 IU/day vitamin D intake significantly reduced the consequent development of Type I diabetes in a survey carried 30 years afterwards, findings which support the hypothesis that vitamin D intake and sunshine inversely correlate with the development of Type I diabetes, rheumatoid arthritis and multiple sclerosis (Hypponen et al. 2001). Six months vitamin D supplementation (1,000 IU/day) increased anti-inflammatory transforming growth factor (TGF-β1) serum levels in multiple sclerosis patients with concomitant reduction in harmful T-cell functions (Mahon et al. 2003). A 3 months trial showed that synthetic $1,25(OH)_2D_3$ reduced the severity of rheumatoid arthritis symptoms (Andjelkovic et al. 1999).

Vitamin D: cardiovascular disease

There is evidence that inadequate vitamin D status and living at higher latitude with concomitant inadequate vitamin D production contributes to the pathogenesis and progression of cardiovascular disease (Zittermann et al. 2005). Clinical studies have reported cross-sectional associations between lower vitamin D levels and plasma rennin (one of most important hormones regulating blood pressure), hypertension, coronary artery calcification, and prevalent cardiovascular disease (Wang et al. 2008). Vitamin D improves vascular muscular function, controls blood pressure, and improves glucose tolerance; whose pathologies are underlying causes of congestive heart failure (Vieth and Kimball 2006). Vitamin D's actions in controlling cytokine immune system signaling molecules assume importance in cardiology through its antiinflammatory actions. A double-blind, randomized, placebo-controlled trial of

daily vitamin D supplementation in congestive heart patients has been found to affect immune-modulating cytokines in desirable ways by increasing serum concentrations of antiinflammatory cytokine interleukin 10 and preventing increases in serum concentrations of proinflammatory cytokine tumor necrosis factor alpha (Schleithoff et al. 2006). These changes indicate that vitamin D has protective effects on the heart and the atherosclerosis that precipitates congestive heart failure, and serves as an antiinflammatory agent for the treatment of this disease as well as other diseases associated with up-regulated proinflammatory cytokines.

Vitamin D: neuroprotection

There is growing evidence that the hormonally active form of vitamin D combats neurodegenerative and neuroimmune disorders by its important role in the central nervous and immune systems and in its promotion of neuronal cell survival (Regulska et al. 2006). Vitamin D assumes importance for normal neural function because the enzymes necessary for $1,25(OH)_2D_3$ production are present in brain tissue (Hosseinpour and Wikvall 2000). There are a plethora of in vitro and in vivo laboratory studies attesting to vitamin D_3-induced neuroprotective effects (Wang et al. 2001). In addition, human studies suggest vitamin D's role in promoting neurological well-being. For example, vitamin D deficiency has been associated with poor cognitive performance in older adults (Llewellyn et al. 2009), while higher serum vitamin D levels have been associated with better cognitive function test performance in older adults including those with Alzheimer's disease (Oudshoorn et al. 2008).

Some of the neuroprotective mechanisms of vitamin D will now be indicated. Vitamin D receptors have been located in multiple brain regions affected by neurodegenerative diseases (Stumpf and O'Brien 1987). As already noted, vitamin D appears to play an important role in autophagy whose disturbance contributes to the pathogenesis of neurodegenerative disorders such as Amyotrophic Sclerosis and Parkinson's, Huntington's and Alzheimer's diseases (Bursch and Ellinger 2005). Autophagy protects against neurodegenerative disorders by enhancing the clearance of mutant aggregate-prone proteins (Ravikumar and Rubinsztein 2006). Decrease in intracellular glutathione content may be related to the primary event in Parkinson's disease, while calcitriol's antioxidant actions have been shown to enhance intracellular glutathione concentration and to protect against reactive oxygen species in the central nervous system (Garcion et al. 2002). Upregulation of glia-derived neurotrophic factors (GDNFs) with consequent antioxidative activity has been proposed responsible for vitamin D_3-induced neuronprotection (Chen et al. 2003). Neurosteroids are a group of steroid hormones synthesized by the brain in the presence of steroidogenic enzymes. Vitamin D_3 in its actions as a neurosteroid has been found to have neuroprotective properties comparable to those of the steroid estrogen in the treatment of some oxidative stress-related neurodegenerative disorders, but without estrogen's undesirable effects (Tetich et al. 2003). There have been some fragmentary reports that vitamin D may play a less positive role as a neuroprotector (e.g., Payne et al. 2008). But these reports are overwhelmed by the plethora of laboratory and human studies indicating that vitamin D combats neurodegenerative diseases.

Vitamin D: macular degeneration

Age-related macular degeneration (AMD), a progressive degenerative condition of the retina, is the most common cause of blindness in the elderly in developed nations (Friedman et al. 2004). Low vitamin D status is associated with the presence of early age-related macular degeneration, with serum vitamin D levels being inversely associated with early AMD (but not advanced AMD), providing evidence that vitamin D may protect against AMD. The ameliorating action of vitamin D may involve the immune responsiveness of drusens, the abnormal extracellular retinal deposits commonly associated with age-related macular degeneration. Since histological studies confirm immune involvement in drusen biogenesis, it has been proposed that vitamin D may protect against AMD by virtue of its immunomodulatory anti-inflammatory properties (Parekh et al. 2007). The antiangiogenic and antiinflammatory roles of vitamin D in combating macular degeneration has already been considered in this review's discussion of vitamin D and angiogenesis.

Vitamin D: some expressed concerns about its effectiveness

As has been presented, there is a substantial body of laboratory and epidemiologic evidence attesting to the salutary effects of vitamin D. Nevertheless, some counterevidence and concerns, albeit fragmentary, have respectively been presented and expressed regarding the effectiveness of vitamin D. While prospective epidemiologic studies of vitamin D status score and pancreatic cancer risk suggest protective associations, a prospective nested case–control study in Finnish male smokers showed a statistically significant threefold increased risk for pancreatic cancer with higher vitamin D status (Stolzenberg-Solomon et al. 2006). Limitations of both prospective and prospective nested case–control studies have later been discussed by the lead coauthor of the Finnish study who concluded that circa year 2009 no conclusions can be made regarding vitamin D's potential role(s) in the etiology of pancreatic cancer (Stolzenberg-Solomon 2009). In an essay it has been suggested that low vitamin D blood levels found in chronic diseases are the result of the disease and not the cause (Marshall 2008). While a plethora of association studies suggest that low 25(OH)D levels contribute to chronic disease, they do not necessarily prove it (association does not necessarily prove causation) and only randomized controlled trials can resolve this conundrum. One such test has been carried out. A randomized double-blind, placebo-controlled trial found that baseline and treatment-induced 25(OH)D concentrations were themselves strong and independent predictors of future cancer occurrence (Lappe et al. 2007). It was reported that the lower the serum 25(OH)D levels, the higher the risk, with improved vitamin D status substantially reducing all-cancer risk in postmenopausal women. In summation, concerns which have been raised about the effectiveness of vitamin D in combating diseases in general and by association those of ageing in particular are as of yet, at the most, only suggestive.

Summary and general conclusions

A review has been presented of the various biological mechanisms and processes by which vitamin D operates and contributes towards positive control of ageing processes. Laboratory and human studies were cited which provide evidence for direct manifestation of vitamin D in general and vitamin D_3 in particular for controlling/ameliorating various ageing pathologies. Some concerns which have been expressed about the effectiveness of vitamin D were also discussed and considered. At this point in time those concerns are judged essentially bereft of substantial basis. Throughout this review emphasis has been placed on relating vitamin D and in particular its potent active metabolite to biogerontology. The evidence presented here strongly indicates that vitamin D's preventative/ameliorating actions appear to be or should play a major positive role in ageing. In conclusion, it is strongly recommended that these actual and probable positive effects be taken into account by gerontologists and biogerontology researchers in their consideration of vitamin D and ageing.

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