Review Article

blood

Vitamin B_{12} deficiency from the perspective of a practicing hematologist

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B_{12} deficiency is the leading cause of megaloblastic anemia, and although more common in the elderly, can occur at any age. Clinical disease caused by B_{12} deficiency usually connotes severe deficiency, resulting from a failure of the gastric or ileal phase of physiological B_{12} absorption, best exemplified by the autoimmune disease pernicious anemia. There are many other causes of B_{12} deficiency, which range from severe to mild. Mild deficiency usually results from failure to render food B_{12} bioavailable or from dietary inadequacy. Although rarely resulting in megaloblastic anemia, mild deficiency may be associated with neurocognitive and other consequences. B_{12} deficiency is best diagnosed using a combination of tests because none alone is completely reliable. The features of B_{12} deficiency are variable and may be atypical. Timely diagnosis is important, and treatment is gratifying. Failure to diagnose B_{12} deficiency can have dire consequences, usually neurological. This review is written from the perspective of a practicing hematologist. (*Blood*. 2017;129(19):2603-2611)

Introduction

Traditionally, vitamin B_{12} deficiency has been considered to lie within the scope and expertise of hematologists. This assignation has deep historical roots, going back to the earliest recognition of the disease that acquired the eponymic title of Addisonian pernicious anemia following the somewhat vague description by the Guy's Hospital physician, Thomas Addison, of "a very remarkable form of general anemia occurring without any discoverable cause whatsoever." It was the astute clinical observations of Richard Cabot, William Osler, and others that brought the picture of the syndromic disease with its classical triad of associated jaundice, glossitis, and myeloneuropathy into sharper focus, as nicely recorded in William Castle's historical review of the disease.[1] Coller,[2] in his commentary to mark the 70th anniversary of *Blood*, wrote: "The most dramatic and far reaching event in hematology in the United States in the pre-*Blood* period was Minot and Murphy's 1926 report that feeding liver to patients with pernicious anemia could cure this otherwise fatal disorder. This dramatic breakthrough was an enormous stimulus to hematologic investigation." A quest for the active principle in liver that made it possible to "cure" pernicious anemia ushered in the era of Big Pharma in a race to identify and produce the compound that ultimately became known as vitamin B_{12}. Elucidation of the physiology of B_{12} and its intricate mechanism of assimilation made it clear that there was a myriad of causes of B_{12} deficiency.[3]

Vitamin B_{12} deficiency: the hematological perspective, past and present

Because of the often conspicuous hematological manifestations of B_{12} deficiency, it remained largely within the domain of hematology. However, as ever more sensitive methods were developed to assess subtle degrees of deficiency of the vitamin,[4,5] it became clear that the effects of B_{12} deficiency were not restricted to the hematopoietic system but were often overshadowed by neurological complications and were sometimes entirely absent.[6] Just as folate deficiency is associated with effects beyond anemia,[7] B_{12} deficiency also can be associated with nonhematological complications, including increased risk of neural tube defect pregnancy, cognitive impairment, osteopenia, and vascular occlusive disease, perhaps attributable to the accumulation of homocysteine (Hcy) that occurs in B_{12} deficiency.[3] Even so, because the most conspicuous manifestations of established B_{12} deficiency affect the blood and bone marrow and are a leading cause of macrocytic and megaloblastic anemia, it is ultimately the practicing hematologist who remains front and center of the clinical diagnosis and management of patients with suspected or confirmed B_{12} deficiency.

This review is written from the perspective of a practicing hematologist who might suspect B_{12} deficiency during a routine patient encounter or who might see a patient in consultation for anemia as part of a complex medical problem. An understanding of the normal physiology and its perturbations in disease is a key factor to the understanding of the causes and manifestations of B_{12} deficiency. The clinical features in a given case of B_{12} deficiency may range from the typical "textbook" picture through any 1 of a kaleidoscopic variety of atypical presentations that can befuddle the unwary.

Pathobiology of B_{12} deficiency

In an adult, the total body B_{12} store is 3 to 5 mg, and the recommended daily intake (RDI) is 2.4 μg.[8] Natural food sources of B_{12} are restricted to food of animal origin. As a consequence, it is a micronutrient that is often in critically short supply, particularly among vegetarian or vegan populations who, through culture, poverty, or conviction, subsist on diets that lack or are poor in B_{12}. Were it not for efficient conservation of biliary B_{12} through enterohepatic reabsorption,[9-11] symptomatic B_{12} deficiency would occur more frequently among vegans.

Complex mechanisms are in place to render B_{12} bioavailable, protect it during intestinal transit, and then absorb and retain the precious vitamin for cellular uptake[3,12] (Figure 1). It is remarkable that B_{12} is the required cofactor for only 2 biochemical reactions in

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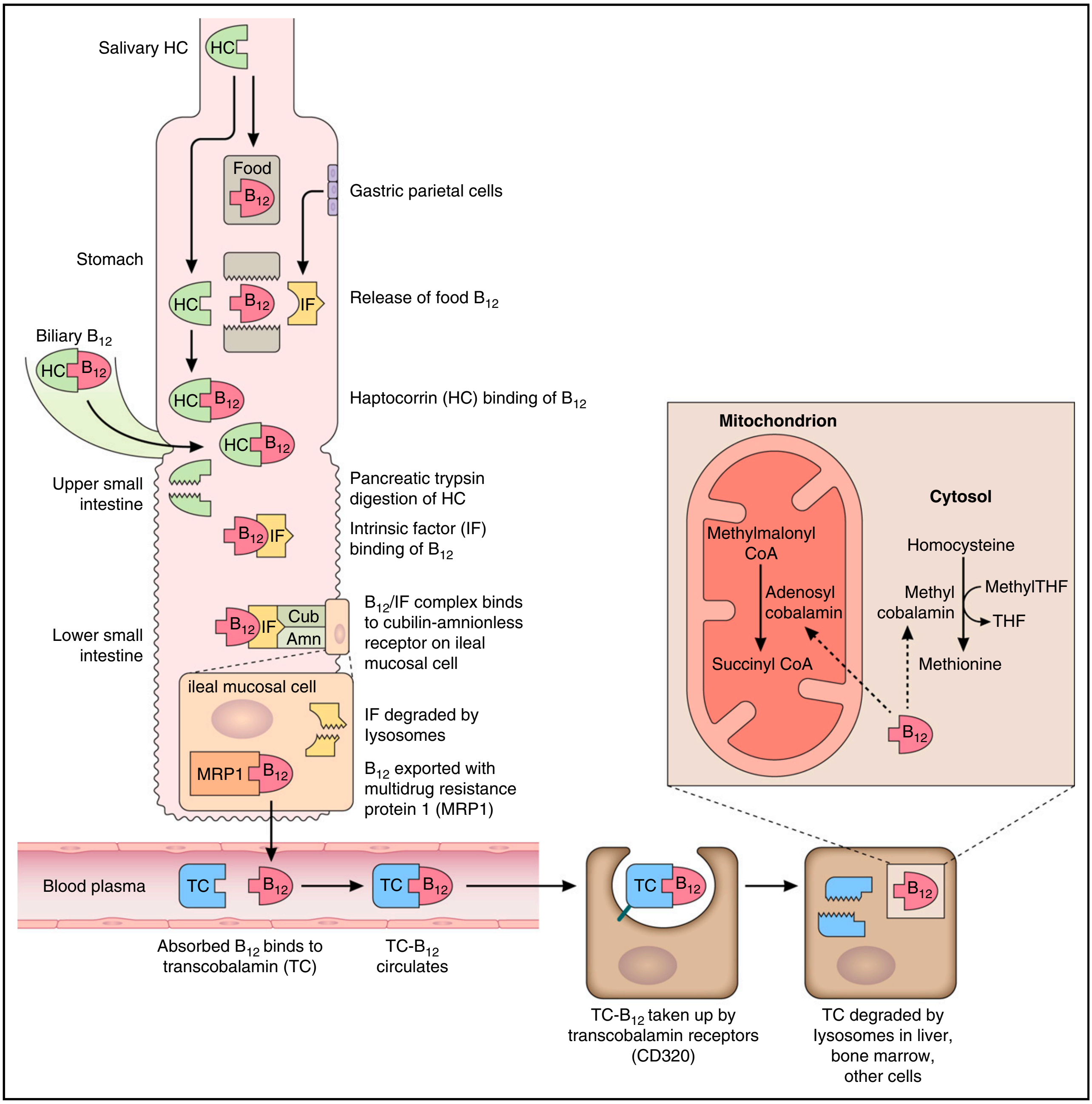

Figure 1. Normal pathway of B_{12} absorption and cellular uptake. Food B_{12} is released in the stomach and binds to salivary HC. In the small intestine, food B_{12} and biliary B_{12} are released from HC by pancreatic proteases and bind to intrinsic factor (IF). The IF-B_{12} complex then binds to the cubam (Cubilin [Cub]–amnionless [Amn]) receptor in the terminal ileum for internalization and release to plasma where it is bound by TC. TC delivers B_{12} to the TC receptor (CD320) on cells, and following release in the cell, B_{12} is reduced and converted to adenosylcobalamin in the mitochondria and methylcobalamin in the cytosol, where they serve as cofactors for the 2 B_{12}-dependent reactions. CoA, coenzyme A; THF, tetrahydrofolate. Professional illustration by Patrick Lane, ScEYEnce Studios.

humans,[3] yet the effects of B_{12} deficiency are not only profound but protean. The several possible reasons for the broad spectrum of manifestations fall into the broad categories of genetic variation and acquired comorbidities.

Depletion of body B_{12} stores resulting from insufficient capture of the vitamin from dietary sources because of either inadequate intake or malabsorption eventually leads to a state of deficiency. When a certain threshold of deficiency is reached, the supply of B_{12} becomes inadequate to support biochemical pathways requiring the vitamin, leading to disruption of the functional and ultimately the structural integrity of cells. Absent of any underlying perturbation of B_{12}-dependent pathways that occur in individuals who harbor inborn errors involving intracellular B_{12} assimilation and processing,[13,14] the major determinant of the severity of B_{12} deficiency and whether it leads to either megaloblastic anemia, demyelinating neurological disease, or both appears to be whether there is abrogation of the normal physiological axis of B_{12} absorption. Normal B_{12} absorption requires intact gastric production of intrinsic factor as well as a functioning cubam receptor for the B_{12}-intrinsic factor complex in the terminal ileum.[3,12,15]

B_{12} and folate are intimately connected through their cooperative roles in one-carbon metabolism, and the hematological complications seen in deficiency of either vitamin are indistinguishable. Both are caused by impaired DNA synthesis that results in a prolongation of the S phase of the cell cycle[16] and causes maturation arrest.[17] Prolongation of the cell cycle is associated with delayed migration of the DNA replication fork and the annealing of DNA fragments synthesized from the lagging strand.[18] The retardation of DNA replication in megaloblasts arises from failure of the folate-dependent conversion of deoxyuridine to deoxythymidine. The deoxyuridine triphosphate that accumulates is incorporated into DNA in place of thymidine 5′-triphosphate by the somewhat promiscuous DNA polymerase enzyme.[19] The normal process of excision-repair of U-A mismatches in DNA fail for persistent lack of thymidine 5′-triphosphate. Repetitive iterations of defective DNA repair ultimately lead to DNA strand breaks, fragmentation, and apoptotic cell death.[20,21]

The morphological appearances of these biochemical lesions are seen as megaloblastic changes in the marrow, which consist of red cell precursors that are larger than normal with a lack of synchronous maturation of the nucleus and cytoplasm (Figure 2). There is a preponderance of basophilic erythroblasts over more mature hemoglobinized forms, creating the appearance of a maturation arrest. The myeloid-to-erythroid ratio falls and may even show reversal (<1:1), due to varying degrees of both apparent erythroid hyperplasia caused by maturation arrest and granulocytic hypoplasia. Megaloblastic features in the granulocyte precursors consist of giant metamyelocyte and band forms containing large horseshoe-shaped nuclei (Figure 2). Megaloblastic megakaryocytes may be seen with abnormally large and polylobated nuclei, sometimes with detached lobes and absent cytoplasmic granulation.

All megaloblastic anemias display similar clinical features. Absent of any sudden acceleration in the rate of B_{12} depletion, such as occurs following exposure to nitrous oxide,[22,23] anemia develops slowly, and symptoms including weakness, palpitations, fatigue, light-headedness, and shortness of breath may not occur until anemia is fairly profound, because compensatory cardiopulmonary changes mitigate tissue hypoxia. The melding of severe pallor with jaundice caused by hemolysis produces a peculiar lemon-yellow skin color.

All formed blood elements are affected by the ineffective megaloblastic hematopoiesis, but erythrocytes show the most marked changes, both in size and in shape, with large oval macrocytes and prominent anisopoikilocytosis. In general, the degree of anemia corresponds with the severity of the red cell morphologic changes. When the hematocrit falls <20%, megaloblasts may appear in the blood. The morphologic features of megaloblastic anemia may be grossly exaggerated in splenectomized patients or in whom there is functional asplenism as occurs in celiac disease or sickle cell anemia when circulating megaloblasts and bizarre red cell morphology may be present.[24]

The anemia is macrocytic (mean corpuscular volume 100-150 fl or more); mild macrocytosis may be the earliest evidence of a megaloblastic process, but because of longevity of red cells, there is a gradual shift in mean corpuscular volume as comingling occurs with older normocytic red cells. Anisocytosis becomes more marked, and the earliest measurable change in red cell indices is an increase in the red cell distribution width.

Neutrophils typically show hypersegmentation of their nuclei, beyond the usual 3 to 5 lobes, and may contain 6 or more lobes.[25] Hypersegmented neutrophils are an early sign of megaloblastosis and may persist for many days after treatment.[25] However, neutrophil hypersegmentation does not appear to be a sensitive indicator of mild B_{12} deficiency.[26] Leukopenia and thrombocytopenia may be present but only rarely cause clinical problems. Thrombocytopenia may be severe, when it may be confused with thrombotic thrombocytopenic purpura.[27,28] Platelet production is reduced to 10% of what would be expected from the megakaryocyte mass,[29] reflecting ineffective thrombopoiesis, and platelets may be functionally abnormal.[30]

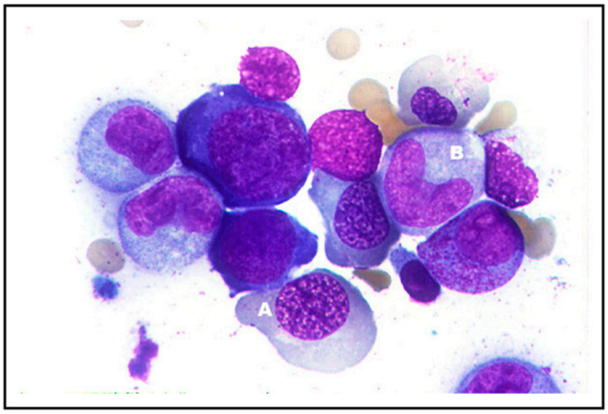

Figure 2. Photomicrograph of bone marrow in a patient with pernicious anemia. (A) Megaloblastic change in the nucleus of an erythroid precursors consisting of variegated finely granular chromatin ("salt-and-pepper" appearance) in contrast to the ground-glass texture of normal proerythroblasts. With progressive maturation, chromatin condensation occurs at a slower pace than normal, giving rise to darker aggregates that fuse nonhomogeneously and impart to the nucleus a characteristic latticelike appearance. Undisturbed maturation of the cytoplasm as hemoglobin forms in a cell with an immature-appearing nucleus results in cells that are conspicuous for their lack of synchrony between nuclear and cytoplasmic development. (B) A megaloblastic ("giant") granulocyte precursor. Original magnification ×100; Wright-Giemsa stain.

Cytogenetic changes, when they occur, are nonspecific and show elongated and broken chromosomes, changes that are usually corrected within 2 days of treatment, although some abnormalities may remain for months.[21,31]

Variations on the theme and the B_{12}-folate nexus

What determines the particular manifestations of B_{12} deficiency in a given individual depends on several factors, some of which are understood, others not. Two clear examples of what influences the clinical presentation in a given patient are the rate of development and the degree of severity of deficiency. The extent to which absorption of B_{12} is compromised, whether partial or complete and whether absorption is totally abrogated or whether it relates only to poor bioavailability of food B_{12} is also important. Polymorphic differences in genes involved in the complex repertoire that comprises the pathways of B_{12} absorption, assimilation, cellular metabolism, and plasma transport of the vitamin (Figure 1) are known to affect the susceptibility to develop B_{12} deficiency.[32,33] Whether these genetic factors can also influence the disease phenotype in B_{12} deficiency is not well understood at this time. Another factor that may play a role in the susceptibility of an individual to B_{12} deficiency is the composition of their gastrointestinal microbiome. Some microbiota are capable of degrading B_{12}, which may affect bioavailability of the vitamin and also lead to the generation of B_{12} analogs.[34] B_{12} analogs have been identified in plasma and tissues[35] and have been invoked as a possible cause of some of the manifestations of B_{12} deficiency.[36] Host-microbial interactions have also been implicated as a possible initiating factor in autoimmune gastritis leading to pernicious anemia. In this proposed mechanism, chronic *Helicobacter pylori* infection may, through molecular mimicry of H^+K^+ ATPase, evoke a host immune response

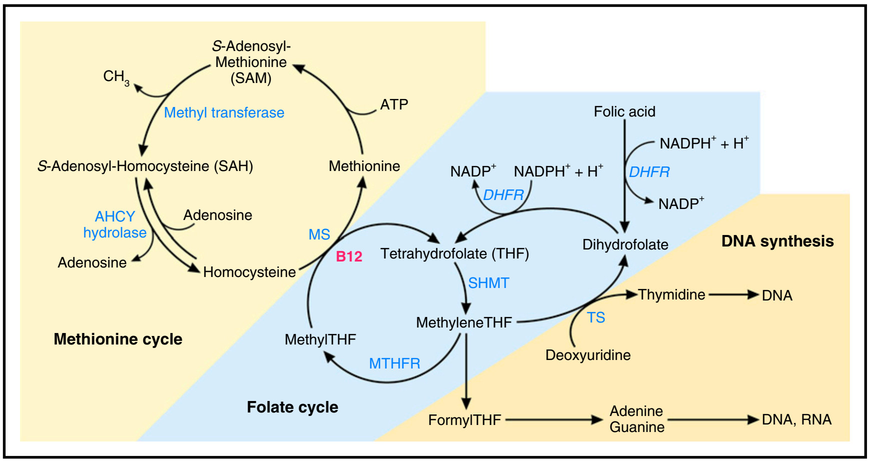

Figure 3. Intersections of B_{12} and folate metabolism, the methionine cycle, folate cycle, and DNA synthesis showing the methyl folate "trap." The key intersection of B_{12} and folate occurs at the methionine synthase (MS) reaction in which the one-carbon methyl group of methyltetrahydrofolate (MethylTHF) is transferred to Hcy to form methionine. The cofactor for this reaction is B_{12} in the form of methylcobalamin. The folate product tetrahydrofolate regains a one-carbon methylene group through the serine hydroxymethyltransferase (SHMT) reaction, and the resulting methylenetetrahydrofolate is essential for conversion of deoxyuridine to thymidine in the thymidylate synthase (TS) reaction. This reaction is rate limiting for DNA synthesis. In B_{12} deficiency, folate becomes trapped as methylTHF. Administration of folic acid can temporarily overcome this block through dihydrofolate reductase (DHFR) reduction to tetrahydrofolate. The other product of the MS reaction, the essential amino acid methionine, after adenosylation to S-adenosyl-methionine (SAM), serves as a universal methyl donor in numerous methyltransferase reactions. The product, S-adenosyl-homocysteine (SAH), undergoes reversible hydrolysis by the enzyme adenosyl-homocysteine hydrolase (AHCY hydrolase), yielding Hcy and thus completing the methionine or remethylation cycle. Not shown in this figure is the alternative transsulfuration pathway for Hcy disposal, which requires vitamin B_6.[8] ATP, adenosine triphosphate; DHFR, dihydrofolate reductase; H^+, proton; MTHFR, methylene tetrahydrofolate reductase; $NADP^+$, NAD phosphate; $NADPH^+$, reduced NAD phosphate. Professional illustration by Patrick Lane, ScEYEnce Studios.

that involves $CD4^+$ T cells through a Fas-dependent mechanism[37] and leads to destruction of the gastric mucosa.[38,39]

Nutrient-nutrient interactions are known to play a role in the manifestations of B_{12} deficiency. The best known of these is concomitant iron deficiency, which can mask the macrocytosis typically seen in B_{12} deficiency. The same is true for other microcytic disorders like α- or β-thalassemia trait.[40,41]

An important B_{12} nutrient interaction is with folate. In B_{12} deficiency, there is disruption of normal folate cycling for regeneration of methylene-tetrahydrofolate, the form required to sustain synthesis of thymidine for DNA replication. Folate becomes effectively "trapped" as methylfolate,[42] because B_{12} is required for its conversion to tetrahydrofolate in the methionine synthase reaction (Figure 3). Trapping of methylfolate creates a state of functional folate deficiency. Supply of folic acid to a B_{12}-deficient patient can intermittently bypass this block through reduction of folic acid to dihydrofolate and then tetrahydrofolate, thereby partially or temporarily alleviating the anemia. Alleviation of the anemia masks the underlying B_{12} deficiency and allows the neurological damage from B_{12} deprivation to continue unabated. There is well-described evidence in the early literature that amounts of folic acid exceeding 1 mg daily given to patients with pernicious anemia were fraught with deleterious outcome.[3,8] Although at first ameliorating hematological features of the disease, even at times with temporary improvement in neurological symptoms, continued administration of folic acid would precipitate or aggravate neurological complications, usually with subsequent recurrence of the anemia.[8,43] Linked to these observations are reports of dissociation between neurological and hematological manifestations in B_{12}-deficient patients[6] as well as an inverse correlation between the degree of anemia and the severity of neurological involvement.[44,45] There is some evidence that this relationship might be related to higher serum folate concentrations in patients with exclusively or predominantly neurological manifestations.[44] More recently, and occurring in the wake of national folic acid fortification programs designed to reduce neural tube defect pregnancies, there have been several reports from longitudinal population studies that individuals with low serum B_{12} levels, who had associated high serum folate levels, had significantly higher levels of methylmalonic acid (MMA) and Hcy and were more likely to show cognitive decline and have lower hemoglobin concentrations than those with low B_{12} but without high serum folate.[46-48] Moreover, individuals with low serum B_{12} and high serum folate had depressed levels of holotranscobalamin (holoTC), indicating an apparent depletion of circulating active B_{12} when serum folate was high.[48] A report that the prevalence of anemia in patients with low B_{12} levels before and after the introduction of folic acid fortification was unchanged argues against the proposition that food fortification may have caused an increase in masking the hematological complications of B12 deficiency.[49] However, it is possible that if there is any deleterious effect of folate in B_{12}-deficient persons, this occurs only in individuals consuming amounts of folate well in excess of the recommended safe upper limit.[8]

Causes of B_{12} deficiency

There are several causes and varying degrees of severity of B_{12} depletion leading to deficiency (Table 1). From the hematological standpoint, it is convenient to divide the causes of B_{12} deficiency into those that frequently lead to megaloblastic anemia or overt neurological complications and those that usually do not.[3,50] The features that distinguish the severe from the mild category of B_{12} deficiency are summarized in Table 2. The separation is based on pathophysiologic considerations and the degree of severity of the deficiency that occurs. The causes that are listed as severe usually involve disease processes that disrupt some aspect of the physiological pathway for B_{12} absorption comprising intrinsic factor and the cubam receptor in the terminal ileum. Undiagnosed or untreated, these conditions ultimately advance to a level of depletion of B_{12} that manifests the clinical features of B_{12} deficiency, either hematological or neurological or both. The exemplar of this category of B_{12} deficiency is pernicious anemia. The slow evolution of this disease reflects the rate at which the autoimmune process disables the manufacture of intrinsic factor in gastric

Table 1. Causes of vitamin B_{12} deficiency

A. Severe deficiency
1. Severe malabsorption (affecting the physiological intrinsic factor cubam receptor axis)
a. Pernicious anemia (autoimmune gastritis)
b. Total or partial gastrectomy
c. Gastric bypass or other bariatric surgery
d. Ileal resection or organ reconstructive surgery (ileal conduit diversion & ileocystoplasty)
e. Inherited disorders affecting B_{12} absorption (affecting either intrinsic factor or the cubam receptor)
2. Abuse of nitrous oxide
3. Inherited metabolic
a. Impaired ability to transport B_{12} (TC deficiency)
b. Impaired ability to process B_{12} (8 distinct inborn errors of cobalamin metabolism resulting in homocystinuria and/or methylmalonic acidemia) with varying clinical spectra involving the nervous system and blood
B. Mild to moderate deficiency
1. Mild to moderate malabsorption (impaired ability to render food B_{12} bioavailable)
a. Protein-bound vitamin B_{12} malabsorption
b. Mild, nonimmune, chronic atrophic gastritis
c. Use of metformin
d. Use of drugs that block stomach acid
e. Chronic pancreatic disease
2. Dietary deficiency
a. Adults: vegans/vegetarian diet, or diet low in meat and dairy products
b. Infants: breastfeeding in infants with vitamin B_{12}-deficient mothers

parietal cells leading to the inexorable depletion of the body B_{12} store. Gastrectomy emulates abrogation of intrinsic factor production but with surgical suddenness. Similar temporal considerations apply in the case of ileal disease vs surgical resection. In the case of chemical inactivation of B_{12} by nitrous oxide, depending on the frequency and duration of its use and the state of B_{12} reserves, deficiency can develop either suddenly or insidiously.[22,23]

The causes of mild B_{12} deficiency, on the other hand, involve either a disruption of the ability to render natural dietary B_{12} bioavailable or a primary dietary lack of B_{12} that is obtained among unsupplemented vegans or, to a lesser extent, among vegetarians.[51] There are several conditions that disrupt the normal processes, as discussed in the review by Nielsen et al[12] and depicted in Figure 1, whereby food B_{12} is rendered bioavailable for absorption through the physiological intrinsic factor-cubam receptor pathway (Table 1). Disruption of the mechanisms to render dietary B_{12} bioavailable all involve a failure of adequate gastric acid production, disrupting the proteolytic activity of peptic digestion.[52] A similar failure of the digestive process applies in the case of chronic pancreatic disease,[53] in which the release of B_{12} from salivary haptocorrin (HC) in the upper small intestine is disrupted through lack of bicarbonate and trypsin production.[54] There are some less common causes of B_{12} deficiency that do not fit nicely into either category, such as infestation with the fish tapeworm, *Diphyllobothrium latum*, in which the degree of deficiency and hence its clinical severity can vary considerably.[55]

Diagnosis of B_{12} deficiency

Two pathophysiologic processes contribute to the anemia resulting from B_{12} deficiency. In addition to the ineffective erythropoiesis caused by intramedullary apoptosis of megaloblastic erythroid precursors,[20] the erythrocytes that are produced have increased rigidity associated with abnormal red cell membrane proteins leading to shortened red cell survival.[56,57] The resulting hemolysis is associated with a 30% to 50% reduction in red cell lifespan. Plasma bilirubin is increased,[58] as is serum lactic dehydrogenase (LDH),[59] with LDH-1 predominating over LDH-2.[60] Serum AST levels are, however, often normal.[61] There is an increase in serum erythropoietin levels, but the increase is relatively modest, compared with other anemias of similar severity.[62] Another feature arising from the ineffective erythropoiesis is a block in iron utilization, resulting in increased serum iron and ferritin levels,[63] but with increased levels of soluble serum transferrin receptor, presumably related to hemolysis.[64] Corresponding to the increase in LDH, there may be an increase in serum muramidase caused by increased granulocyte turnover.[65]

Serum B_{12} levels

Although often used as the first-line screening test for B_{12} deficiency, serum B_{12} measurement used in isolation has a generally poor sensitivity and specificity for reliable detection of B_{12} deficiency.[4,5] A low serum B_{12} level does not always indicate B_{12} deficiency, and a serum B_{12} within the reference range does not always connote normalcy. There are several reasons serum B_{12} is not low in all patients with B_{12} deficiency. In part, this relates to the distribution of B_{12} on the 2 major plasma B_{12} binding proteins. Normally, the major fraction (70% to 90%) of circulating B_{12} is bound to HC, which is unavailable for immediate delivery to cells. The other 10% to 30% is bound to transcobalamin (TC), the functional B_{12} transport protein. Consequently, if levels of the HC-bound fraction are conserved, the total serum B_{12} level may lie within the normal reference range, despite lowered levels of the important TC-bound fraction. An extreme example of this is seen in a B_{12}-deficient patient with normal serum B_{12} levels who has an underlying myeloproliferative disease with high HC levels.[66] In almost 50% of patients with low vitamin B_{12} levels, levels of the biochemical markers, MMA and Hcy, were found to be normal, and these patients had no hematologic or neurologic response to B_{12} replacement therapy, suggesting that the

Table 2. Severe and mild categories of B_{12} deficiency

	Severe	Mild
Mechanism	Disruption of intrinsic factor/cubam absorption	Failure of gastric digestion and release of food B_{12}
Enterohepatic reabsorption of biliary B_{12}	Interdicted	Intact
Manifestations	Megaloblastic anemia and/or neurological complications	Megaloblastic anemia and serious neurological deficits rare; associated with more rapid cognitive decline
Rate of depletion	Rapid, and may be extreme	Slow, usually mild and usually limited
Treatment	Require lifelong regular B_{12} replacement, either monthly injection or daily high-dose oral B_{12}	Responds to daily physiological dose supplements of oral B_{12}

low B_{12} values were false positive results.[67] Serum B_{12} levels are usually normal in functional B_{12} deficiency, resulting from exposure to nitrous oxide, which chemically inactivates the methylcobalamin at the active site of the methionine synthase during its catalytic cycle.[68] Serum B_{12} levels are also usually normal in TC deficiency, and inborn errors of cobalamin metabolism.[69] Conversely, serum B_{12} levels may be low in the presence of normal tissue B_{12} in vegetarians,[70] in subjects taking megadoses of ascorbic acid,[71] in inherited "benign" HC deficiency,[72,73] and in a substantial proportion of patients with megaloblastic anemia resulting from folate deficiency (30%).[4]

Serum holoTC

The B_{12} bound to HC comprises 70% to 90% of the total plasma B_{12}, yet is unavailable for cellular delivery. That TC is the critical plasma B_{12} binding protein is underscored by the fact that inherited TC deficiency is associated with serious hematological and neurological sequelae and, if untreated, fatal outcome,[74] whereas HC deficiency has no morbid consequence.[73] Theoretically, measurement of the TC-bound fraction of the plasma B_{12} (holoTC), also termed "active B_{12},"[75] should be more relevant for assessing functional B_{12} status, even though it constitutes only 10% to 30% of the total plasma B_{12}. Increasingly, holoTC measurement is being used for clinical assessment of B_{12} status, either singly[3,76,77] or in combination with the total serum B_{12} with or without measurement of the metabolites MMA and Hcy.[78-80] In addition, holoTC levels also reflect B_{12} absorptive capacity.[81-83]

Serum or plasma MMA and Hcy

Because they are the substrates of the 2 B_{12}-dependent reactions, elevated levels of MMA and Hcy are sensitive indicators of tissue B_{12} deficiency. Their levels are high in >90% of B_{12}-deficient patients and increase before serum B_{12} falls to subnormal levels.[4,5] Even when there is no manifest evidence of clinical B_{12} deficiency, and serum B_{12} levels are not low, elevated levels of MMA and Hcy can be considered as sensitive biomarkers of a subclinical underlying state of B_{12} deficiency, which may potentially progress to a state of manifest B_{12} deficiency with its attendant clinical complications that may remain subtle, often being only neurological[84] or may become more exuberant.[3,85,86] MMA measurements can be carried out on either plasma or serum, whereas Hcy is best measured in plasma, because cellular release of Hcy in a clotted blood sample can alter Hcy levels.[87,88] Elevated plasma MMA and/or elevated Hcy are both indicators of B_{12} deficiency in patients without impaired renal function or an inherited defect in cobalamin processing enzymes.[4,13,14,89] Of the 2, MMA measurement is both more sensitive and more specific, and elevated MMA will persist for several days, even after B_{12} treatment. MMA elevation is seen only in B_{12} deficiency, unlike Hcy levels that also increase in folate and pyridoxine deficiencies, as well as in hypothyroidism.[4] However, certain intestinal microbes synthesize propionate, a precursor of MMA, and when there is bacterial overgrowth in the small intestine, as occurs in blind loops following gastrointestinal surgery, microbial-derived MMA may contribute to elevations in plasma MMA.[5,90] Although measurement of these metabolites may be used in population screening for B_{12} deficiency, the finding of an isolated elevation of plasma MMA should not be taken as proof of clinically attributable B_{12} deficiency, absent of any ancillary measurements to support that diagnosis or any demonstration of a therapeutic response to the administration of B_{12}.[90,91]

Assays of B_{12} absorption and intrinsic factor antibodies

There is currently no approved test in routine clinical use to measure B_{12} absorption since the Schilling test became obsolete. Lack of a validated B_{12} absorption test hampers accurate diagnosis of pernicious anemia as the cause of B_{12} deficiency and clinical investigations related to all causes of B_{12} malabsorption.[92] One possible test that shows promise, the Cobasorb test, is based on the measurement of the change in holoTC following oral administration of nonradiolabeled cobalamin.[82,93,94] An alternative approach has been described using accelerator mass spectrometry to quantify ^{14}C in the blood following an orally administered dose of [^{14}C]-cyanocobalamin.[95]

In absence of any test for B_{12} absorption, definitive diagnosis of pernicious anemia is problematic and depends on the demonstration of circulating antibodies to intrinsic factor and gastric parietal cells. Antibodies to intrinsic factor can be of 2 types, varying according to the epitope on the intrinsic factor molecule to which they are directed. For diagnostic purposes, the so-called "blocking" type, directed against the B_{12} binding site, is measured, as this type not only is highly specific for pernicious anemia but also is the species present in 70% of patients.[96] Antibodies to parietal cells, although present in 90% of patients with pernicious anemia, are less specific, as they can occur in simple atrophic gastritis and in autoimmune thyroid disease.[97]

Prevention and treatment of B_{12} deficiency

Regarding prevention of B_{12} deficiency, the Institute of Medicine Food and Nutrition Board has defined the RDI for adults at 2.4 μg daily but with the caveat that individuals 51 years and older obtain most of this amount through consuming foods fortified with B_{12} or in a B_{12}-containing supplement.[8] This rider is added in consideration of the high prevalence of food B_{12} malabsorption caused by gastric dysfunction among the elderly. Assuming that the lowest possible MMA level is consistent with optimal well-being, a large segment of the population may exist in a state of precarious B_{12} balance, as evidenced by the fact that concentrations of serum MMA leveled off to a nadir in healthy individuals consuming 4 to 7 μg B_{12} daily.[98] One of the possible implications of this finding is that individuals consuming less B_{12} may have a narrow margin of safety in the event that they were to develop any condition that further compromised their state of B_{12} repletion. Provided the physiologic intrinsic factor–cubam pathway for physiologic B_{12} absorption is intact, a daily supplement of B_{12} of 10 μg or more would suffice to prevent B_{12} deficiency or to maintain adequate B_{12} status in individuals with food B_{12} malabsorption caused by gastric dysfunction, including atrophic gastritis or the chronic use of drugs that impair acid production, such as proton pump inhibitors.[12,50] The defined RDI notwithstanding, it is important to recognize that individuals with pernicious anemia or any other condition that interdicts the physiological intrinsic factor cubam absorption pathway would not benefit from the additional Institute of Medicine recommendation.

It is worth noting that prospective interventional trials using Hcy-lowering vitamin supplements containing B_{12} in subjects at high risk through suboptimal baseline B vitamin status show a slowing of cognitive decline and of cerebral atrophy.[99] Considering that vitamin B_{12} deficiency is the dominant modifiable cause of hyperhomocysteinemia in the post–folic acid fortification era,[100] it is reasonable to conclude that B_{12} adequacy is important to maintain, and this becomes progressively more relevant with advancing age.

Concerning treatment of confirmed B_{12} deficiency, well-defined guidelines have been enunciated,[50,101] the details of which still apply. Some important principles need emphasizing. Where the cause of the deficiency is not known or irreversible, treatment must be lifelong. In general, the form and dosage of treatment depend first on whether the intrinsic factor-dependent pathway is intact or not. If not intact, then the choices lie between intramuscular injection of 1000 μg B_{12} (cyanocobalamin in the United States; hydroxocobalamin in Europe) given every other day for 1 to 2 weeks followed by weekly injections for a month and then tapered to once a month indefinitely. Only ~10% of each B_{12} dose is retained. The alternative to injected B_{12} is high-dose oral B_{12}. Between 1% and 4% of an oral dose of B_{12} is absorbed passively, even when the intrinsic factor-dependent pathway is abrogated.[102] Consequently, oral replacement therapy with B_{12}, which was used successfully in the past,[103] has again come into vogue,[104] because of convenience and cost. In most instances, however, it would be prudent to "top up" a B_{12}-deficient patient through parenteral injection before switching to the oral route for maintenance, with due vigilance concerning compliance, particularly in the elderly. Because the passive route of absorption of B_{12} applies to all mucosal surfaces, approved sublingual and intranasal formulations of B_{12} are also available. It should be noted that patients with pernicious anemia at times report that the recommended treatment schedule is not adequate to relieve all their neurological symptoms and therefore often request, or may even treat themselves with, B_{12} injections more frequently than the guidelines suggest. No biological basis for this apparent increased requirement for B_{12} replacement is known, but because there are no reports of adverse effects associated with excess B_{12} intake, there is no reason to advise against this practice.[8]

Conclusion

Although considered an "old" disease, new information is constantly accruing about B_{12} deficiency, the broad array of its effects, and methods for its diagnosis. B_{12} deficiency primarily affects the hematopoietic system, but its effects extend to other tissues and organs, most notably the nervous system. The spectrum of clinical presentations is broad so that diagnosis depends first on a high index of suspicion and then on the judicious application of appropriate testing. Because B_{12} deficiency is amenable to simple replacement therapy, diagnosis is critical. Several questions still remain unanswered concerning B_{12} deficiency, including the possible harmful effects of high folate levels in subjects with low B_{12} status, particularly with respect to neurological damage. Other newer areas of investigation that may provide better insights into the variability of expression of B_{12} deficiency include genetic analysis and the effects of the microbiome.

Authorship

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