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Is innervation an early target in autoimmune diabetes?

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In the non-obese diabetic (NOD) mouse, a spontaneous model of type 1 diabetes (T1D), recent evidence suggests that Schwann cells (Scs) and neurons surrounding insulin-producing β cells of the islets of Langerhans are destroyed before β cells. During normal perinatal development, macrophages (MΦ) are involved in phagocytosis of apoptotic neurons. Pertinently, MΦ are already present at birth in NOD pancreata. Their possible abnormal control of nerve phagocytosis, together with transient β-cell hyperactivity and lymphocyte anomalies, might conjointly participate in T1D pathogenesis.

Type 1 diabetes (T1D) is thought to result from autoimmune β-cell destruction. Despite extensive investigation, mainly in non-obese diabetic (NOD) mice, the etiology of T1D remains unknown [1]. However, recent data support the notion that innervation is an early target in its pathogenesis, with β-cell targeting occurring later [2,3]. The first immune cells, MΦ and dendritic cells (DCs), also known as antigen-presenting cells (APCs), accumulate around NOD islets and ducts at weaning (three weeks of age) (Figure 1a). Subsequently, T cells migrate to the pancreas and home around ducts (periductulitis) and islets (peri-insulitis), followed by MΦ, DCs and T-cell infiltration into the islets (insulitis) (Figure 1b). Destructive insulitis coincides with scavenger MΦ influx and leads to β-cell destruction [1].

Are β cells the only targets in T1D?

The fact that β cells are thought to be specifically targeted is puzzling in light of: (i) the peri-insular location of the first infiltrating immunocytes because β cells are located in the center of the islet and the islet blood flow follows a β → α direction [4] (Figure 1a and Figure 2a); (ii) the periductular infiltrate, which develops concomitantly with peri-insulitis and insulitis (Figure 1b); (iii) the undisturbed progression of insulitis in NOD mice, whose β cells lack MHC class I, with no or little progression to hyperglycemia [3]; (iv) the non–β-cell specificity of some islet-cell antigens (ICAs), which include glutamate decarboxylase (GAD); (v) the various autoantibodies (anti-GAD, anti-ICA512 and anti-glima 38) directed against proteins shared by islet and neuronal cells [5]; and (vi) the appearance of autoantibodies to GAD before those to

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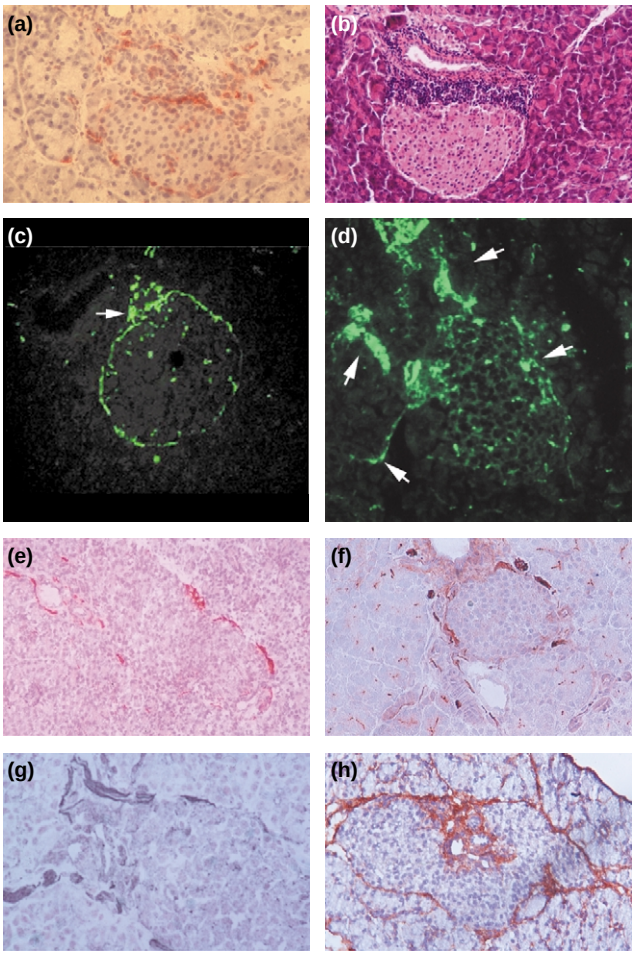

Figure 1. Various aspects of immune-cell infiltration and islet structure, particularly in relationship with innervation, suggesting that Schwann cells (Scs) and nerves might be earlier targets than β cells in autoimmune diabetes. **(a)** After weaning, at one month of age, antigen-presenting cells (APCs), for example, $CD11^+$ dendritic cells (DCs), converge on the peri-islet and -ductular areas of the non-obese diabetic (NOD) pancreas (original magnification ×400). **(b)** Later, as shown here in a two-month old NOD pancreas, lymphocytes similarly infiltrate the islet, inducing peri-ductulitis, peri-insulitis then insulitis (original magnification ×400). **(c)** Data confirming this hypothesis showed that glial fibrillary acidic protein $(GFAP)^+$ Scs, which are considered trophic support of the peripheral nervous tissue (PNS), envelop the islet at its periphery and converge, forming a neuro–insular complex (NIC) (arrow), seen in this six week-old NOD pancreas but also in control strains (reproduced with permission from Ref. [2]). **(d)** In a four- or five-week old NOD mouse pancreas, GAD immunoreactivity in nerves and NIC (arrows) is stronger than in β cells; in NOD, but not in control, mice it is surrounded by lymphocytes and then disappears. Reproduced with permission from Ref. [19]. *Copyright 1996. The Endocrine Society.* **(e)** and **(f)** Peripherin and neurofilament 200, other markers of peripheral islet innervation, are shown here in NOD*scid* pancreata at two and three weeks of age, respectively (×400). **(g)** During early postnatal pancreas development, innervation is the only Fas^+ structure at the ductular–insular pole and islet periphery, as shown in a two-week old NOD mouse pancreas. Reproduced with permission from Ref. [16]. **(h)** The extracellular matrix protein, collagen I, is localized at the ductular–insular pole and islet periphery, forming the islet basement membrane, as shown in this two-week old NOD pancreas (×400). Clearly, Sc and nerves line this basement membrane, which will be broken to allow macrophage and lymphocyte influx into NOD islets.

other β-cell antigens and preceding T1D onset [5]. It is worth noting here that GAD autoantibodies were first discovered in the central nervous system (CNS) disease, Stiff-Man syndrome, and that β cells and neurons share, to some extent, similar gene expression patterns and cellular processes [5–7].

The nervous system is not spared in mice with the NOD genetic background

Autoreactive T cells, which routinely target classical islet and CNS antigens, have been found in NOD mice, diabetic patients, their relatives with high diabetic risk and multiple sclerosis patients [8,9]. Moreover, the immune system in NOD mice lacking the co-stimulatory molecule B7-2 shifts to overtly attacking peripheral nerves, inducing a chronic inflammatory demyelinating polyneuropathy with only mild insulitis [10]. Mice with the NOD genetic background are somewhat sensitive to experimental acute encephalomyelitis induction, either specifically or, more surprisingly, nonspecifically [11,12].

Intriguingly, pre-diabetic NOD and even NOD*scid* (severe combined immunodeficiency) mice show hippocampal astrocyte changes. Indeed, when one month-old NOD and NOD*scid* mice are hyperinsulinemic with low glycemia, their hippocampi have more glial fibrillary acidic protein ($GFAP^+$) astrocytes (e.g. astrogliosis) than controls [1,13]. Astrocyte numbers rise further after diabetes onset. Thus, astrocytosis might be an adaptive response to protect neurons against hypo- or hyperglycemia. Astrocytes also produce various substances, for example, cytokines, growth factors, prostaglandins and nitric oxide, which can modify the brain microenvironment [14] and stimulate glial MΦ differentiation into immature DCs, which will mature in the presence of lymphocytes [15]. Hence, as suggested, astrocytes might act as APCs and trigger (auto)immune processes in the NOD CNS.

Pancreatic innervation is a precocious actor in T1D

The pancreatic nerve network is dense in periductular and peri-islet areas, precisely where the first infiltrating APCs are observed (Figure 1a and Figure 2b), respectively [16,17]. Postnatally, neuro–insular complexes (NICs) (Figure 1c) partially regress, as does sympathetic innervation in the CNS and peripheral nervous system (PNS), a process that involves apoptosis and MΦ [2,16]. Throughout life, sympathetic [γ-aminobutyrate (GABA) and GAD-containing], cholinergic and peptidergic nerves, complexly regulate islet hormone release [18].

In NOD and control mouse pancreata, as in rats, neurons and β cells express GAD [18,19] (Figure 1d and Figure 3). Moreover, in four- or five-week old NOD pancreata, lymphocytes infiltrate around GABAergic, GAD-containing fibers. Then, these nerves disappear rapidly in NOD females but persist in control mice [19]. After weaning, when transient β-cell hyperactivity starts, autoantibodies to peripherin, a marker of developing innervation (Figure 1e), and T-cell reactivities against peripherin and both GAD isoforms appear in NOD mice [1,20–22]. Pertinently, an early, selective (e.g. not seen in exocrine tissue) and marked loss of sympathetic nerves is observed in and around the islets of BioBreeder rats, another spontaneous model of T1D, at diabetes onset [23]. Streptozotocin-treated rats do not show this alteration. These data suggest that peri-islet and -ductular nerves, which are also identified by neurofilament 200 expression (Figure 1f), might be a precocious actor in T1D pathogenesis.

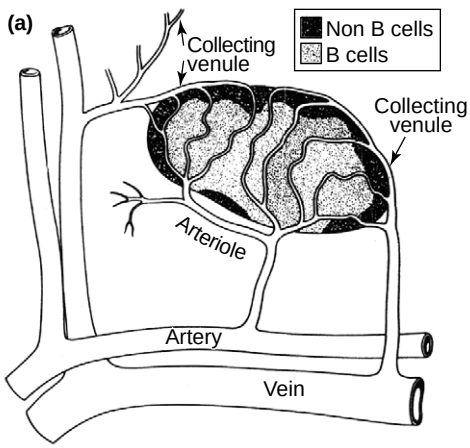

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Figure 2. Vascularization and innervation of the islet of Langerhans. **(a)** Compartmentalization of β versus non-β cells and islet microvasculature (reproduced with permission from Ref. [4]) showing: (i) that β cells are situated in the center of the islet and surrounded by other endocrine non-β cells, that is, α, δ and pancreatic polypeptide cells, and (ii) the organization of vascularization, which would normally enable circulating immunocytes to directly access β cells (if they were the first targets) and not the islet periphery. **(b)** Innervation of the islet of Langerhans: the large nerve trunk at the vascular–ductular pole gives rise to the peri-insular ganglia (p.i.g.), the peri-insular plexus and the 'neural terminal' net in and around the islet. Reproduced with permission from Ref. [17]. Compared to Figure 1a and 1b, the first infiltrating cells would be more likely to be in contact with peri-islet nerves than central islet β cells.

Regarding pancreatic innervation, Fas^+ fibers are present from birth onwards even in control mice, in peri-islet, -ductular and -vascular areas, where Fas ligand $(FasL)^+$ structures are particularly concentrated [16] (Figure 1g). Fas and FasL expression by neurons and Schwann cells (Scs) might contribute to physiological neuron loss, especially that of sympathetic innervation during development (for references therein, see Ref. [16]). Perinatally, CNS and PNS neuron apoptosis are associated with the presence of MΦ [24]. What normally happens to perinatal pancreas innervation is unknown. Many scavenger MΦ are present in neonatal pancreata but their numbers progressively decline until one month of age [25]. Concomitantly, nerves expressing interferon (IFN)-induced protein-10 (IP-10) are surrounded by MΦ, suggesting that this chemokine might attract leukocytes for nerve remodeling [16].

In the PNS, $GFAP^+$ Scs are the counterpart of CNS glial astrocytes. In the pancreas, Scs envelop the islet at its periphery, as if delimiting the islet and exocrine parenchyma [2,26,27] (Figure 1c). Axons are usually in contact with the outer Sc surface but sometimes creep beneath them. Sc-produced extracellular matrix (ECM) acts as an organizer of peripheral nerve tissue and strongly influences Sc adhesion, growth and differentiation and regulates axonal growth [26]. To interact with the ECM, Scs express numerous integrin and non-integrin surface receptors [26]. Figure 1h illustrates that collagen, Scs and neurons are similarly located. In one-month old NOD females, T cells are attracted to the endocrine–exocrine junction, where they accumulate [2]. Then, T cells penetrate into the Sc mantle and usually concentrate contiguous to it, rather than moving into the central islet β cells. Scs progressively disappear before β-cell deficiency appears and one-month old NOD females already have autoreactive T cells and autoantibodies specific to GFAP. By contrast, Scs are not destroyed in control mice, NOD*scid* mice or after chemically induced β-cell damage [2]. Injection of GFAP-specific T-cell lines into NOD*scid* mice alters the Sc envelope but does not induce β-cell destruction and diabetes [2]. Moreover, IFN-γ modifies Sc surface MHC class I and intercellular adhesion molecule-1 (ICAM-1) clustering and upregulates MHC class I, II and ICAM-1 expression and, notably, Sc autoimmunoreactivity exists in patients with inflammatory neuropathies [27,28]. Thus, Scs might be early targets or have an immunoregulatory role in PNS alterations, linked or not to T1D.

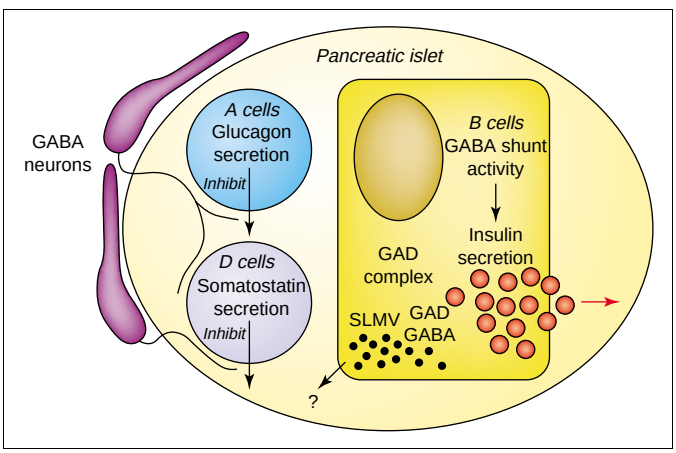

Figure 3. This scheme emphasizes the localization of the γ-aminobutyrate (GABA)-ergic, glutamate acid decarboxylase (GAD)-containing, innervation at the islet periphery and its connection with endocrine cells, as described in rats (reproduced with permission from Ref. [18]).

How to reconcile autoimmune targeting of nerve components with that of β cells in T1D

Figure 4 presents a scenario, based on the existence of serial victims, which might solve the T1D 'mystery murders' [29]. NOD MΦ exhibit various anomalies, especially reduced phagocytic ability, which can perturb apoptosis [16,30]. Normally, apoptosis does not trigger an inflammatory response but in certain circumstances, apoptotic cells induce an immune reaction that can

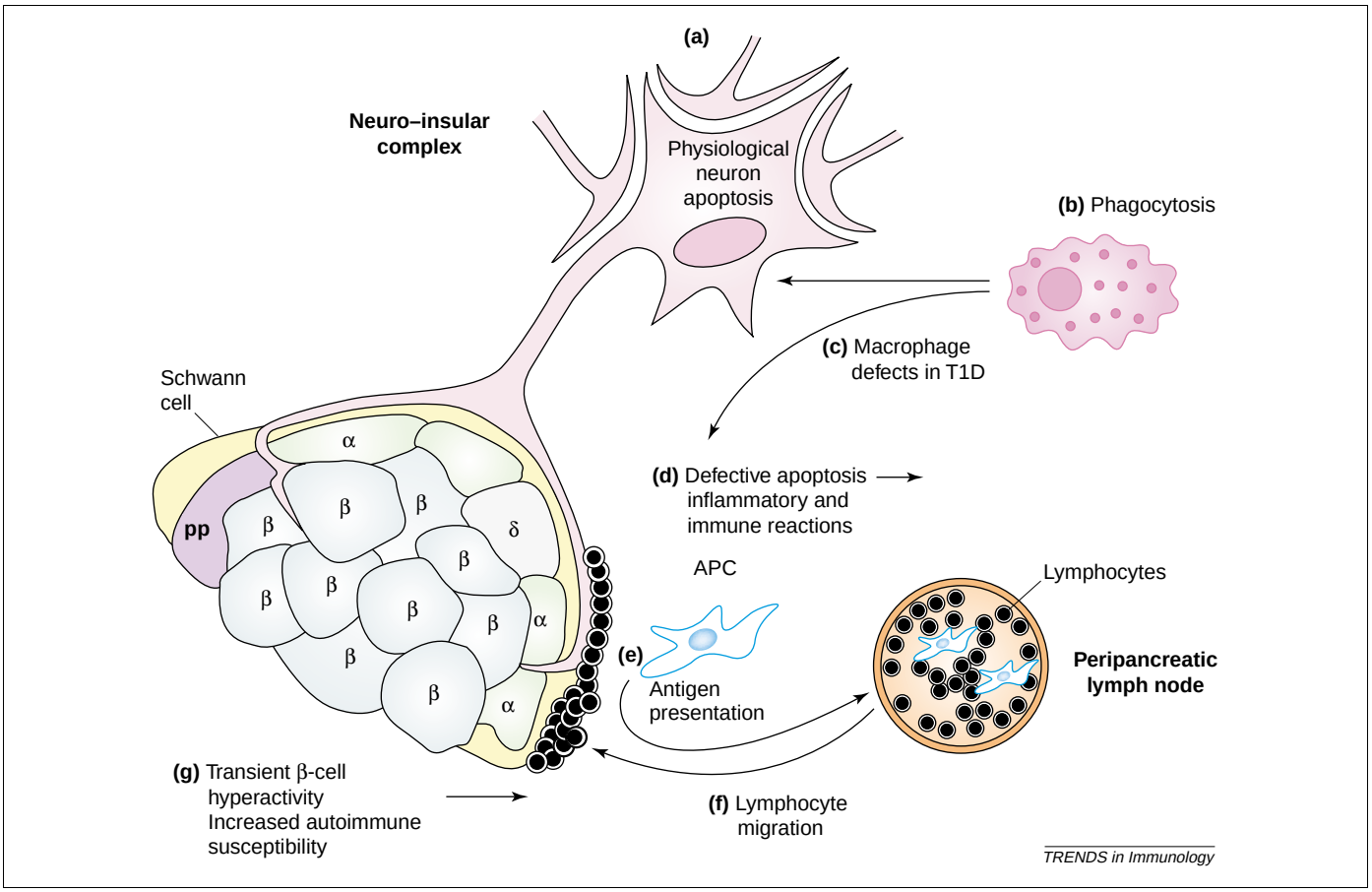

Figure 4. Hypothetical scenario attempting to solve the 'murder mysteries' of autoimmune diabetes in non-obese diabetic (NOD) mice. **(a)** Physiological degeneration of Fas^+ and glutamic acid decarboxylase $(GAD)^+$ neuro–insular complex (NIC) and peripheral islet innervation would occur perinatally. **(b)** Macrophages (MΦ) would normally eliminate apoptotic neurons. **(c)** NOD mouse MΦ have several defects in type 1 diabetes, particularly defective apoptosis [30]. **(d)** These defects can induce an inflammatory reaction adjacent to neurons and Schwann cells (Scs), triggering cytokine production. **(e)** Damaged neurons and Scs are abnormally phagocytized by MΦ, and processed antigen- and MHC class II-expressing antigen-presenting cells (APCs) would activate T cells in peripancreatic lymph nodes. **(f)** Here, this scenario rejoins that of Kaufman [29]: once activated, lymphocytes migrate to and accumulate around the islet periphery (peri-insulitis); the presence of abundant innervation in the periductular area and/or the existence of a common ductal precursor for endocrine and exocrine cells might explain why periductulitis develops [7]. T cells synthesize cytokines, particularly interferon-γ (IFN-γ), propagating the autoimmune reaction that intensifies GAD-containing neuron and Sc destruction before that of endocrine cells. Then, breaches form in the islet basement membrane, enabling lymphocyte infiltration (insulitis) and β-cell targeting [2]. **(g)** β-cell hyperactivity would increase susceptibility to the autoimmune reaction [1].

increase susceptibility to (or development of) autoimmunity [31]. The hypothesis, that MΦ abnormalities might disturb developmental organ remodeling favoring autoimmunity, is reinforced by observations in the salivary gland, another target in NOD mice [32]. In neonatal pancreata and salivary glands, FasL expression is abnormally high when Fas^+ nerves and MΦ are also present. Abnormally controlled perinatal nerve degeneration could explain the early presence of various antibodies and autoreactive T cells directed against neurons and Scs in NOD mice. T1D-prone individuals and diabetic patients also have antibodies and T-cell autoreactivity to Scs [8]. Furthermore, NOD salivary hypofunction is associated with autoantibodies and T-cell reactivity with neuron-specific reactivity [33].

In addition to MΦ and DCs, lymphocytes are present in developing human pancreas, and might also be involved in tissue remodeling and/or self-tolerance [34]. Moreover, they exhibit many anomalies in human and rodent T1D [35]. Still concerning lymphocytes, one should note that, by comparing NOD mice to NOD*scid* or *rag* (recombinase-activating gene)-deficient mice, events triggered or not by lymphocytes can be distinguished. Moreover, despite their being highly inbred, NOD mice, prone to polyendocrine autoimmunity and CNS immune disease, enable the spontaneous evolution of these diseases and their potential relationships(s) to be studied. By contrast, transgenic models might bypass the earlier natural history events and 'enter' the disease by a different mechanism.

Finally, the GAD autoantibody–epitope shift coincides with diabetes onset and might result from target-cell change [36]. In addition to neurons, endocrine non-β cells that might be damaged to some extent can express GAD, thereby partly explaining the non-β-cell specificity of ICAs [5]. However, as peripheral islet autoimmunity progresses, β cells would finally become the 'target' because they share various antigens with nerves, they are hyperactive due to genetic and/or environmental factors and they have a major role in glucose homeostasis [1,37]. Hyperactive β cells express more autoantigens, adhesion and MHC molecules, produce neo-epitopes (proinsulin) and are more sensitive to cytokine-induced damage: these phenomena increase their

autoimmune susceptibility [1]. This scenario is compatible with recent data showing that the interaction of β-cell class I with MHC class I-restricted T cells is not necessary for the initiation or early progression of insulitis but is important for the late switch from benign to malignant insulitis [3]. Moreover, glucose abnormalities in NOD mothers (regardless of their origin) might downregulate fetal insulin secretion through sympathetic nerve activation. Later, during postweaning NOD hyperinsulinemia, the same downregulation might recur, suggestive of hyperactivity of the 'primary' target (e.g. innervation).

Concluding remarks

T1D, mainly observed in children and young adults, is a multigenic disease strongly dependent on numerous environmental factors that can influence each islet component from fetal life onwards [38]. T1D might originate from defective physiological developmental events concerning one component, such as islet innervation, involving dysfunctional MΦ and lymphocytes. Analysis of cytokine, growth factor and chemokine expression patterns during pancreas development might be informative. The intricate morphological interaction between developing nerves and islet cells and/or their precursors, the existence of various genes shared by nerves and islet cells, particularly *BETA2* (also known as *NeuroD2*), which induces neuron and β-cell differentiation, are intriguing and deserve further investigation [17,39]. Polymorphisms in the *BETA2* (*NeuroD2*) gene have been linked to T1D (for references therein, see Ref. [1]). Moreover, another candidate recently proposed as an 'early' autoantigen, the islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), might maintain the 'suspense' [40,41]. Finally, examination of the localization and immunological role of GAD67, the preponderant isoform in the mouse compared to rats and humans, might be revealing [42]. Later during the diabetogenic process, genetic abnormalities [possibly in relationship with the *IDDM2* (insulin-dependent diabetes mellitus) locus] and/or environmentally induced functional β-cell abnormalities (discussed elsewhere, Refs [1,38]) and their resemblance to nerves would make β cells particularly sensitive to the autoimmune reaction and designate them as the only obvious victims.

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Letters

Vitamin C, respiratory infections and the immune system

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Almost a century ago, several authors suggested that vitamin C might affect respiratory infections. However, not much attention was paid to this topic until 1970, when Nobel laureate Linus Pauling wrote the bestseller 'Vitamin C and the Common Cold.' His claim that gram-dose vitamin C supplementation would prevent and alleviate colds was not based on any studies of his own but on previously published trials. One result of his activity was that a series of placebo-controlled trials was carried out to determine whether large doses of vitamin C would affect colds [1–3]. The new trials found that ≥ 1 g day^{-1} vitamin C supplementation had no consistent effect on common cold incidence (Figure 1). Consequently, these trials did not support the suggestion that regular vitamin C ingestion would increase the resistance of the general Western population to colds. However, some evidence indicated that vitamin C could have moderate preventive effects in restricted groups, such as subjects with particularly low dietary intake or those suffering from acute physical stress [1,3].

However, the placebo-controlled trials found that the duration and symptoms of colds were reduced by ≥ 1 g day^{-1} vitamin C, although, the quantitative results diverge sharply (Figure 1). In most of the trials, the decrease in morbidity was between 5% and 35%, with a mean of 23%. Evidently, the main question should not be to decide whether a decrease of 23% is clinically important but to identify the factors that could affect the magnitude of the benefit. For example, even in the gram-dose region, there is a trend for trials with 2–4 g day^{-1} doses to show greater benefit when compared to trials using 1 g day^{-1} [1,2]. All trials summed up in Figure 1 used regular daily vitamin C supplementation. If the main goal is to alleviate the symptoms, it appears more rational to administer vitamin C therapeutically, starting immediately after the early symptoms; however, few such trials have been carried out.

Vitamin C could also affect lower respiratory tract infections. Several early reports suggested that vitamin C might hasten convalescence from pneumonia, a hypothesis that was supported by one placebo-controlled trial [3]. Three controlled trials with human subjects reported a significantly lower incidence of pneumonia in vitamin C supplemented groups [3,4], suggesting that under certain conditions, vitamin C might affect susceptibility to pneumonia. Studies with guinea pigs and other animals have also found that vitamin C modifies susceptibility to various viral and bacterial infections, including pneumococcal infections [3,4]. Recently, a new coronavirus was identified as the cause of the severe acute respiratory syndrome (SARS), and two reports of vitamin C studies are of particular interest in this regard. Vitamin C increased

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