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M2-like cells from the macrophage lineage might play a central role in closure of the embryonic neural tube

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A B S T R A C T

Herein it is hypothesized that M2-like macrophages or pre-macrophages of fetal origin might play a central role in development and closure of the neural tube. Early in embryonic development, pre-macrophages arise from the fetal yolk sac and track through the bloodstream to reach diverse embryonic tissues, where they mature. Most of these macrophages exhibit an M2-like phenotype. The critical period for neural tube closure is contained within the period of yolk sac-derived pre-macrophage tracking and distribution, which poses a question: might these pre-macrophages or macrophages exert an influence on the closing neural tube? Evidence suggests that perturbations in macrophage polarization or M2 macrophage function might contribute to the failure of neural tube closure associated with diabetes mellitus, one carbon metabolism (including folic acid deficit), inositol, arachidonic acid, and sphingosine-1-phosphate, as well as in the teratogenicity of nitric acid, valproic acid, and fumonisin. The influence of each of these factors is interpreted in light of potential interactions with M2-like macrophages or macrophage progenitors on the developing neural tube. By placing these anti inflammatory macrophages at the center of various epigenetic, neurochemical, and signaling processes suspected to be involved in neural tube closure, potential associations are revealed between macrophages and embryonic structural developmental processes such as collagen and actin dynamics. The choice of this model is also an attempt to explain why some etiologies for failure of neural tube closure are rescued by folic acid, whereas other etiologies are rescued only by formate, inositol, or not at all.

Introduction

It was observed in the 1970s that pregnant mothers of fetuses with neural tube defects (NTDs) were more likely to exhibit deficiencies of folic acid, riboflavin, and vitamin C then were control mothers of healthy fetuses [1]. This observation lead to the discovery of maternal folic acid supplementation as an effective means of prevention for most neural tube defects. Since the 1970s, extensive research has been devoted to various clinical and epidemiological aspects of folic acid supplementation, as well as to the potential of other nutritional supplements in preventing NTDs.

Despite the gains on the clinical front, both risk factors and preventative factors for NTDs remain poorly understood, and many basic questions have yet to be answered. For example, how does folic acid promote normal neural tube closure? Why might hyperglycemia be teratogenic to the developing neural tube? On account of the complex interplay of factors which appear to influence neural tube closure — including vitamins, co-factors, and substrates for one carbon metabolism; factors in the cytosol and factors in the mitochondria; factors involved in cell signaling; and factors involved in neurotransmission — many of the mechanisms underlying this process remain elusive. One candidate player that may be involved in many of these diverse processes is the yolk sac derived macrophage or pre-macrophage.

During early embryonic development, pre-macrophages arise from the fetal yolk sac and traverse the embryonic vascular network to penetrate diverse fetal tissues. Most of these fetal derived macrophages are polarized to the M2 or M2-like phenotype. In contrast to myeloid cells derived from sites such as liver or bone marrow, which do not arise until after normal closure of the caudal neuropore, yolk sac derived pre-macrophages arise and track to tissues during a span of time which is concurrent with neural tube closure. Some remain in a pre-macrophage state during this period, whereas others differentiate into mature macrophage morphology [2–4].

Some evidence suggests that macrophages or macrophage like cells may play a role in embryonic differentiation. In the frog, macrophage migration inhibitory factor (MIF) is expressed in high levels in embryonic neural tissue. When MIF is knocked down and macrophages migrate elsewhere, gastrulation proceeds normally whereas neurulation is inhibited. The implication is that macrophages must play a role in the neurulation process, at least in some animal models [5]. Using this inference as a springboard, the discussion herein poses the question: Might macrophages play a role in neural tube closure? And if so, which macrophages?

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Hypothesis

Much work has been done to identify exogenous factors which influence closure of the neural tube, yet little is known about the mechanisms which underlie these effects. Factors known to influence neural tube closure are diverse: they impact on mechanisms ranging from one-carbon metabolism, to cell signaling, to actions of chemokines and neurotransmitters on gene expression. It is tempting to conceive of a central mechanism which might unify, in some way, all of these diverse interactions. Such a mechanism would need to influence or else to be influenced by each of the various processes identified by protective or teratogenic factors; and it would also need to connect those processes to the building and maintenance of the developing neural tube. It is with this goal in mind that the M2 anti-inflammatory macrophage — or, more precisely, the M2-like macrophage or macrophage progenitor — is proposed as a key player in the development and closure of the neural tube. After all, macrophages are already known play a role in tissue remodeling, regulation of actin, and interactions with collagen: actions which are potentially critical for neural tube development.

Three main categories of influencers on neural tube closure are discussed herein with respect to associations with macrophage mediated processes. One category includes influencers of one carbon metabolism such as folic acid, choline, betaine, formate, vitamin B-2, vitamin B-6, and vitamin B-12. All of the above factors are involved in one-carbon metabolism. One-carbon metabolism, in turn, enables methylation of DNA, a process which may be required for macrophage polarization to the anti-inflammatory M2 phenotype. Folate itself also interacts with cell surface receptors and is involved in macrophage interactions with collagen. Since collagen plays a critical structural role in the developing neural tube, this category of factors — the category involved in one-carbon metabolism — illustrates a potential web of interactions between nutritional factors, M2 macrophages, and structural processes occurring within the developing neural tube.

A second category of influencers on neural tube closure includes the signaling molecules inositol triphosphate (IP3) and sphingosine-1-phosphate (S1P). It also includes fumonisin, a pro-apoptotic teratogen for the neural tube which interferes with sphingolipid metabolism. Both IP3 and S1P are involved in interactions with macrophages: IP3 promotes macrophage survival, activation and chemotaxis, and S1P prevents macrophage apoptosis and also promotes macrophage polarization to the M2 phenotype. The ability of inositol to rescue NTDs unresponsive to folic acid might relate to inositol's upstream regulation site on macrophages. Whereas folic acid influences macrophage polarization and enhances M2 macrophage function, IP3, which influences macrophage survival, activation and chemotaxis, acts on the general macrophage pool.

A third category of influencers on neural tube closure includes the teratogens nitric oxide, diabetes mellitus, and valproic acid, as well as sildenafil, a pharmacological agent which rescues from valproic acid induced NTD's. Nitric oxide accumulation sequesters vitamin B12, a necessary cofactor for one-carbon metabolism, thereby potentially contributing to defects in methylation during development. Furthermore, accumulation of nitric oxide may result in production of peroxynitrite, a potent oxidizing agent which promotes apoptosis in macrophages and is an inducer of NTD's. Diabetes mellitus promotes the production of nitric acid by macrophages and other cells, and it also reduces Pax3, a regulator of the pro-apoptotic tumor suppressor p53 which promotes macrophage polarization to the M1 phenotype. Valproic probably causes NTDs *via* its inhibition of the NMDA receptor, whose actions play a role in both IP3 production and p53 regulation. Macrophages might oppose the action of valproic acid: through their release of glutamate, macrophages are capable of stimulating the NMDA receptor. Sildenafil, a cGMP phosphodiesterase inhibitor, might rescue from NTDs either *via* its cGMP-related action on peroxynitrite accumulation, or *via* its cGMP-related stabilization of the nitric oxide pool.

In summary, out of all the exogenous factors known to influence risk for NTD's, most of them also appear to be capable of influencing the M2 macrophage pool. As a trend, preventative factors for NTD's tend to increase M2 macrophage activity or increase the overall macrophage pool, whereas factors teratogenic for neural tube closure tend to decrease M2 activity or reduce the macrophage pool. In light of a known role for macrophages in the development of many tissues, and in light of a role for macrophages in the regulation of structural building blocks of neurulation such as actin and collagen, these associations suggest a key role for M2-like cells of the macrophage lineage in neural tube closure.

Evaluation of the hypothesis

General basis for the hypothesis

Of the factors known to influence risk for NTDs, most are capable of influencing the M2 macrophage pool. In general, preventative factors for NTDs tend to increase M2 macrophage activity by promoting polarization of macrophages toward the M2 phenotype, by promoting M2 macrophage function, or by increasing the overall macrophage pool. Risk factors for NTDs tend to decrease M2 activity either by promoting macrophage polarization away from the M2 phenotype or by reducing the macrophage pool.

Preventative factors and risk factors are detailed below, along with the various mechanisms by which each of the factors influence macrophage survival, chemotaxis, or polarization to the M2 phenotype.

Folic acid and one carbon metabolism

Folate, a preventative factor for NTDs, is a potent inducer of both M2 macrophage polarization and M2 macrophage function (See Fig. 1).

Folate is integral to the synthesis of methionine and S-adenosine methionine (SAM), a critical substrate for methylation reactions involving DNA, RNA, and protein synthesis; DNA methylation; and synthesis of a wide variety of other molecules critical for life. DNA methylation has been implicated as having a role in neural tube closure. In macrophages, DNA methylation is required for polarization to the M2 phenotype in several tissues. It is possible that DNA methylation might be required for M2 polarization in the embryo, as well [6–10].

It is known that addition of folate reduces the accumulation of

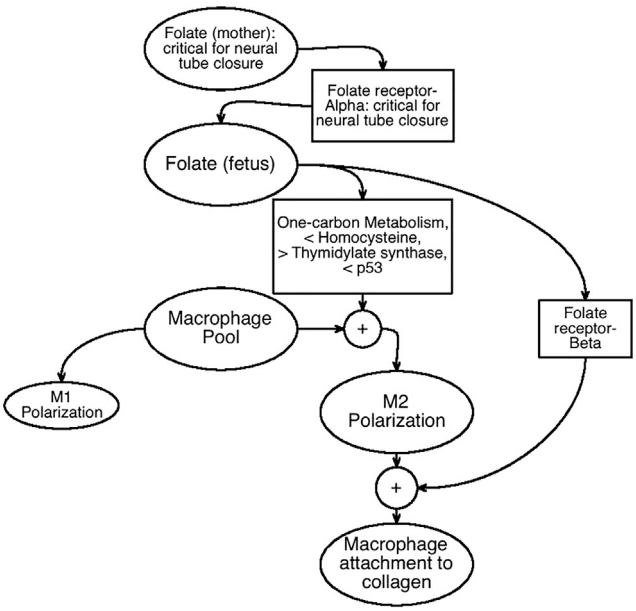

Fig. 1. Folate interactions with macrophages.

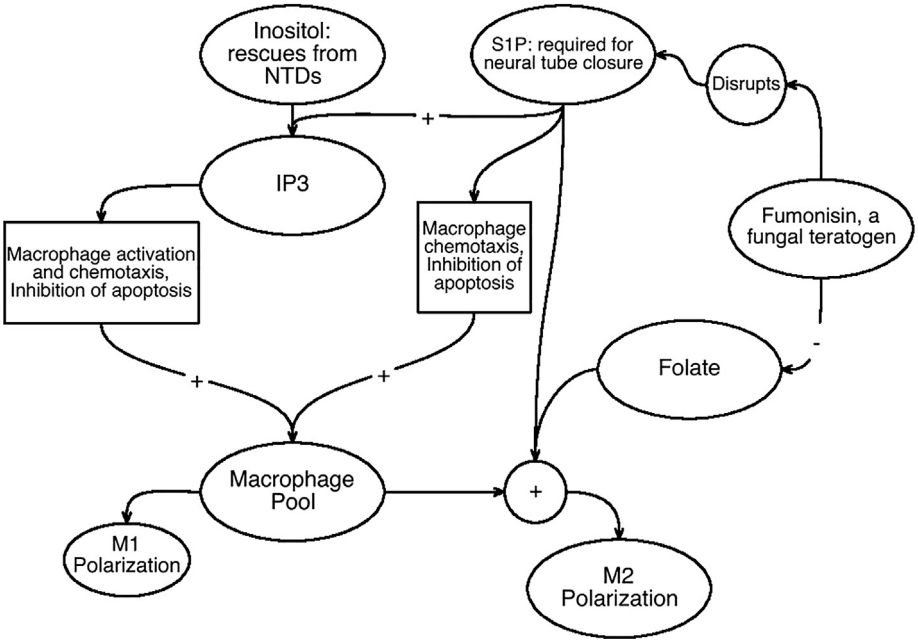

Fig. 2. IP3 and S1P interactions with macrophages. (IP3: inositol triphosphate. S1P = sphingosine-1-phosphate).

homocysteine by enabling the methionine synthase reaction. This mechanism was previously thought to contribute to NTDs. The homocysteine hypothesis fell from favor when it was found that, under conditions of methionine deficit, supplementary homocysteine actually prevented NTDs — presumably by enabling methylation. However, it is possible that homocysteine might increase the risk for NTDs under special circumstances.

Homocysteine is a proinflammatory molecule, and it promotes polarization to the M1 rather than the M2 phenotype. In healthy individuals, intermedin appears to protect against extremes of homocysteine induced pro-inflammatory macrophage polarization. In diabetes mellitus, however, intermedin is reduced, and homocysteine likely exerts a more significant effect on macrophage polarization toward M1 and away from M2 [5,11–13]. If the hypothesis is correct that macrophage polarization influences the risk for NTDs, then in the context of diabetes mellitus, homocysteine might be a risk factor rather than a neutral or preventative factor.

Folate also activates the macrophage folic acid receptor beta (FR-beta). FR-beta is expressed only in activated macrophages, and it is expressed preferentially on the cell surface of macrophages polarized to the M2 phenotype. The M2 macrophage FR-beta receptor regulates macrophage attachment to collagen — a mechanism which should be of interest given that collagen IV is necessary for formation of the caudal neural tube in the mouse embryo, and collagen XVIII is necessary for neural tube closure in humans [14–18]. Thus, the action of folate on M2 macrophages and its regulation of macrophage attachment to collagen could be a necessary step in the formation and closure of the neural tube.

In addition to FR-beta, folate also activates the folate receptor alpha (FR-alpha), a receptor which is expressed on the cell surface of placental cells and other tissues. FR-alpha is involved in maternal-to-fetal transport of folate across the placenta, thus providing the fetus a source of maternal folate for methylation reactions. As noted above, in macrophages, DNA methylation is required for polarization to the M2 phenotype in several tissues, though this phenomenon has not yet been studied in the embryo. FR-alpha appears to be important for neural tube closure: in women with inhibited binding of folic acid to FR-alpha, the risk for NTDs is increased [19].

Another potential mechanism for the influence of folic acid on macrophages is its upregulation of thymidylate synthase (TS), an enzyme which catalyzes the folate dependent reductive methylation of dUMP to dTMP. Folate-induced increases in TS lead to decreased expression of the tumor suppressor p53, whose activation is associated with downregulation of macrophage M2 gene expression [20–22]. Thus, by upregulating TS and down regulating p53, folic acid may promote polarization of macrophages to the M2 phenotype.

In addition to folate, a number of other cofactors and substrates for one-carbon metabolism are thought to reduce the risk for neural tube defects. These factors include choline, betaine, formate, vitamin B-2, vitamin B-6, and vitamin B-12. Choline, betaine, and formate are important sources of one-carbon units; and vitamins B-2, B-6, and B-12 are important cofactors in one carbon metabolism [23–25]. Presumably these factors prevent NTD's for some of the same reasons that folic acid prevents NTD's: they enable methylation. Incidentally, these factors also reduce homocysteine, a molecule which might contribute to the development of NTD's under certain specific circumstances. As with folic acid, it is likely that the other factors involved in one-carbon metabolism increase macrophage polarization to the M2 phenotype, at least to some degree: they do so both via DNA methylation and via reductions in homocysteine. Among preventative factors involved in one-carbon metabolism, formate is a special case, in that it diffuses freely across the mitochondrial membrane in both directions. By entering mitochondria, formate can prevent NTD's associated with mitochondrial defects which are resistant to folic acid [25].

A role for the signaling molecules inositol trisphosphate (IP3) and sphingosine-1-phosphate (S1P)

This section addresses IP3 and S1P, two signaling molecules which exert effects on macrophage activation, on macrophage polarization to the M2 phenotype, and on the process of neural tube closure (See Fig. 2).

Inositol, a building block for the signaling molecule inositol trisphosphate (IP3), is carbocyclic sugar with no direct relationship to one-carbon metabolism. Inositol has a number of active phosphorylated forms which are involved in signal transduction in a variety of tissues. In mouse macrophages, the signaling molecule IP3 is involved in macrophage activation and chemotaxis in the presence of platelet activating factor, an activating factor for a variety of immunological cells. IP3 also inhibits apoptosis via induction of Bcl-xL, an anti-apoptotic

factor expressed in macrophages and myeloid precursors. For inositol to exert its preventative effects on NTDs, it must first be phosphorylated to IP3. This conversion depends on arachidonic acid and protein kinase C; without either arachidonic acid or specific isoforms of protein kinase C, inositol appears unable to exert its preventative effect [26–29].

S1P is a signaling molecule involved in the regulation of both immunological cell function and embryonic development. S1P is involved in embryonic development of the cardiovascular and central nervous systems, and in the sphingosine kinase-null mouse embryo, S1P deficiency disrupts neural tube closure. In macrophages, S1P is anti-apoptotic and also a chemoattractant; and extracellular generation of S1P promotes macrophage polarization to the M2 phenotype. The actions of S1P on neural tube closure might relate in part to the actions of IP3: in endothelial cells, the action of S1P receptor on calcium release from the endoplasmic reticulum depends on the inositol trisphosphate pathway [30–32].

A role for S1P and other sphingolipid derivatives in neural tube closure was highlighted by a 1990–1991 outbreak of NTDs at the Texas-Mexico border, in Mexican-American women whose stable was corn. The outbreak was caused by fumonisin, a fungal mycotoxin which contaminates corn. Fumonisin is a pro-apoptotic ceramide synthase inhibitor which induces accumulation of bioactive intermediates of sphingolipid metabolism and also depletes complex sphingolipids. This action of fumonisin on sphingolipids appears to interfere with the placental folate transporter FR-alpha, thereby potentially depriving the fetus of folate. Furthermore, by altering levels of ceramide and sphingosine, fumonisin alters the balance between S1P and ceramide [33–35].

Together, IP3 and S1P promote macrophage survival, activation, chemotaxis, and polarization to the M2 phenotype. Both IP3 and S1P are necessary for closure of the embryonic neural tube. It is of interest that inositol, which has been reported to rescue from NTDs in conditions unresponsive to folic acid, appears to increase M2-like macrophages via a mechanism upstream of that of folic acid [36]. While folic acid and other factors involved in one-carbon metabolism stimulate macrophage M2 polarization and enable M2 macrophage function, IP3 is capable of stimulating macrophage activation and chemotaxis at a locus further upstream, at the level of survival, activation, and chemotaxis for the general pool of macrophages.

Teratogens which interfere with closure of the neural tube

This section addresses the interactions between macrophages and teratogenic factors known to increase the risk for NTDs, including diabetes mellitus, nitric oxide, and valproic acid. The fungal teratogen fumonisin has been addressed in the previous section (See Fig. 3).

Diabetes mellitus is a proinflammatory state which is characterized both by increased nitric oxide and by increased polarization of macrophages toward the pro-inflammatory phenotype. In diabetes mellitus, as proinflammatory macrophages and other immune cells release nitric oxide and reactive nitrogen species into the local hyperglycemic environment, nitric oxide exerts a teratogenic effect on the developing neural tube [37,38]. How does nitric oxide produce NTDs? One possibility relates to the biphasic action of nitric oxide on macrophages and other cells.

At lower concentrations, nitric oxide serves a protective function for the developing neural tube and also protects macrophages from apoptosis. At higher concentrations, however, nitric oxide reacts with superoxide anions (O2·-), resulting in the formation of peroxynitrite (ONOO–), a powerful oxidising agent. It turns out that peroxynitrite is pro-apoptotic in a variety of cells, including macrophages [39–41]. Through the actions of peroxynitrite, the elevated levels of nitric acid found in diabetes mellitus might induce apoptosis both in cells of the developing neural tube, as well as in cells such as macrophages or macrophage precursors.

Another mechanism of nitric oxide teratogenicity might be related to its interaction with vitamin B12. Since nitrite, a metabolite of nitric oxide, is capable of sequestering vitamin B12, nitric oxide excess might create a local deficiency of vitamin B12 within the embryo [42]. Since vitamin B12 plays a critical role in one carbon metabolism, a local deficiency of vitamin B12 might be expected to increase the risk for NTDs.

Folic supplementation rescues from nitric oxide induced NTDs [43], a finding which is consistent with both the peroxynitrite theory and the vitamin B12 theory for teratogenicity for the neural tube: it is consistent with the peroxynitrite theory because folic acid is an antioxidant, and it is consistent with the vitamin B12 theory because folic acid facilitates one-carbon metabolism and methylation.

Diabetes mellitus probably interferes with neural tube closure via multiple pathways in addition to nitric oxide. One important pathway involves decreases in Pax3, possibly via abnormalities in the Wnt/catenin signaling pathway. Pax3 is a protein which suppresses p53 dependent cell death during embryonic development of the neural tube. As hyperglycemia inhibits Pax3 expression, the resultant reduction in Pax3 leads to increased expression of p53, a pro-apoptotic tumor suppressor protein. P53 most likely increases the risk for NTDs at least in part on account of its pro-apoptotic behavior. p53 also inhibits macrophage polarization to the M2 phenotype, again suggesting a potential role for M2-like cells of macrophage lineage in these processes [44,45].

Another pathway which may be involved in the teratogenesis of diabetes mellitus involves its increased production of long-chain acyl-CoA synthetase 1 (ACSL1), an enzyme that catalyzes the thioesterification of fatty acids. ACSL1 is a pro-inflammatory effector which promotes polarization of macrophages toward a more inflammatory phenotype – once again illustrating a potential connection between macrophage polarization and neural tube development [46].

Valproic acid (VPA), an anticonvulsant medication and a teratogen for the developing neural tube, apparently exerts its teratogenicity on the neural tube via perturbation of NMDA receptors important for neural tube closure. The NMDA receptor is a glutamate receptor present in multiple tissues throughout the body. In the embryo, the NMDA receptor is involved in several actions which influence on the development of the neural plate [47].

In animal models, the NMDA receptor modulates calcium dynamics in the developing neural plate. Exposure of embryos to a specific NMDA receptor antagonist results in excessive proliferation and decreased migration of neural plate cells, thereby changing the geometry of the neural tube and resulting in NTDs. The teratogenic action of VPA on the NMDA receptor mirrors the teratogenic action of a NMDA receptor antagonist on the receptor: both the specific receptor antagonist and VPA interfere with glutamine signaling via the NMDA receptor, and the teratogenic results also mirror one another. Exposure of embryos either to VPA or to a specific NMDA receptor antagonist causes abnormalities in calcium signaling, cellular proliferation, and geometric orientational migration, all of which may result in NTDs [47]. How does NMDA receptor modulation of calcium dynamics influence cellular behavior within the neural plate? One candidate mediator is ERK1/2, a signaling molecule recruited by glutamate signaling which is involved in the activation cellular proliferation [47].

A role for macrophages in influencing the NMDA mechanism for neural closure has not been well clarified. One idea is that, in the embryo, macrophages or pre-macrophages might secrete glutamate and thereby activate NMDA receptors in the neural plate. *In vitro*, macrophages produced glutamate which stimulated NMDA receptors [48]. It is possible that in the embryo, too, macrophages might interact with NMDA receptors and thereby influence neural tube development.

Another mechanism for NMDA receptors in neurulation might relate to interactions of the NMDA receptor with arachidonic acid. Agonists of the NMDA receptor produce a dose dependent release of arachidonic acid from neurons; and exposure of neurons to arachidonic acid potentiates NMDA currents [49,50]. As noted in the previous section, arachidonic acid one of the factors which is necessary for conversion of

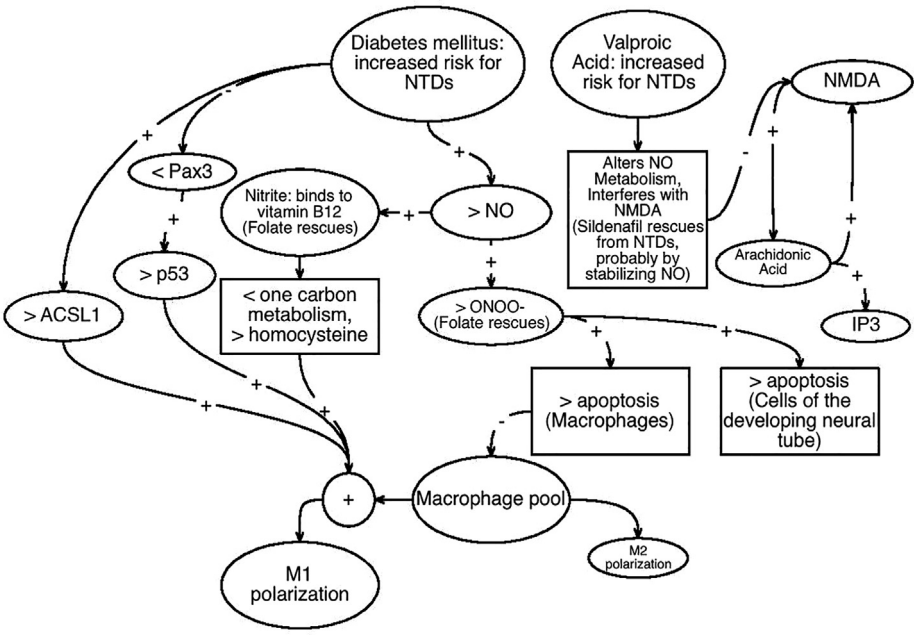

Fig. 3. Selected teratogenic influences. (ACSL1 = long-chain acyl-CoA synthetase 1).

inositol to IP3, a signaling molecule critical for neural tube development which also influences macrophage survival and chemotaxis. Perhaps NMDA receptors influence neural tube development via increases in arachidonic acid, thereby potentiating IP3 signaling.

One clue to another potential action of valproic acid and NMDA receptors on the developing neural tube relates to the rescue of VPA induced NTDs by sildenafil, an inhibitor of cGMP phosphodiesterase 5 [51]. As a cGMP phosphodiesterase inhibitor, sildenafil increases cGMP, which forms complexes with nitric oxide. By complexing with nitric oxide, cGMP might reduce the availability of nitric oxide for oxidation to peroxynitrite. This relationship is suggested by the observation that cGMP protects human macrophages against peroxynitrite-induced apoptosis [40]. Alternatively, cGMP might prolong nitric oxide in tissue, thereby minimizing nitric oxide deficit.

Does VPA increase or decrease nitric oxide? The answer is not clear. Inhibition of nitric oxide synthesis enhances the teratogenicity of VPA, which suggests that VPA may act as a nitric oxide depleting agent. On the other hand, in the presence of interferon gamma and mycobacteria, VPA actually increases nitric oxide [52]. Perhaps sildenafil rescues from VPA induced teratogenicity by protecting nitric oxide both from depletion and also from oxidation to peroxynitrite. Such a mechanism could enable sildenafil to protect against both the highs and lows of nitric oxide. And under conditions of excess, sildenafil could protect macrophages against peroxynitrite-induced apoptosis.

It has been observed that high doses of nitric oxide — enough for significant production of peroxynitrite — inhibit the NMDA receptor, whereas lower doses of nitric oxide do not inhibit the NMDA receptor [53]. Therefore, it is is possible that sildenafil rescues VPA induced teratogenesis both by attenuating peroxynitrite induced inhibition of the NMDA receptor and also by attenuating peroxynitrite induced apoptosis in macrophages. Given that nitric oxide teratogenicity for the neural tube is rescued by folic acid, it may come as no surprise that, in mice, VPA induced teratogenicity to the neural tube is also rescuable by folic acid [54,55].

Interactions between M2 macrophages and structural components of the developing neural tube

To demonstrate that M2-like macrophages play a role in influencing neural tube closure on a structural level, one would need to demonstrate that M2-like macrophages are capable of exerting effects, either directly or indirectly, on factors which contribute to the structural integrity of the developing embryo. Known factors which influence structure and geometry of the neural tube include actin, collagen, integrin, and many other factors. For at least two of these factors – actin and collagen – direct or indirect relationships to macrophages have been demonstrated.

Actin is a critical structural component for the developing neural tube: it is required in the process by which the two neural folds become fused. As the neural folds approximate one another to fuse, epithelial cells in the midline develop actin-rich protrusions which form the first points of attachment between the two neural folds. In the mouse, early neurulation is characterized by “filopodia,” or spike-like protrusions, whereas late neurulation and neural tube closure is characterized by “ruffles,” or sheet like lamellipodia [56].

Some evidence suggests that macrophage activity may relate to the regulation of actin deposition and organization within the developing neural tube. For example, IP3, which, as noted above, is an important protective agent for macrophages, also interacts with actin in the process of protrusion geometry. This is evidenced in mice by the observation that when Rac1, a small GTPase which interacts with IP3 and the actin cytoskeleton, is deleted, the cells in late neurulation exhibit “filopodia” rather than “ruffles.” The result in these mice is a low spina bifida. However, IP3 and Rac1 are not only critical structural regulators for actin: they are also involved in the facilitation of monocyte migration, as is evidenced by the observation that suppression of Rac1 by interferon gamma results in decreased monocyte migration [57,58]. The reasons and implications for Rac1 facilitated macrophage migration during neurulation remain unclear.

With respect to actin, perhaps a primary role for macrophages and pre-macrophages relates to expression of the fractalkine receptor CX3CR1. Both pre-macrophages and macrophages express CX3CR1, and in a myeloma cell line, fractalkine-CX3CR1 interactions were found to stimulate actin enriched membrane protrusions and ruffling [2,59]. This interaction between CX3CR1 seems promising, but further studies are needed in the context of the developing embryo.

Fractalkine itself may modulate neurulation: in the hippocampus it potentiates the NMDA receptor, and by interacting with CX3CR1 on macrophages and pre-macrophages, it also serves as an anti-apoptotic agent for these immune cells [60,61]. The actions of fractalkine on the

NMDA receptor and on macrophages might help to explain why the fetuses of some, but not all, women exposed to VPA develop NTD's.

Another molecule involved in the structural integrity of the developing neural tube is collagen. Collagen IV has been implicated in development of the neural plate, and disruption in the gene coding for collagen XVIII results in neural tube defects. Both folic acid and macrophages appear to be involved in the regulation of collagen deposition and organization, as is evidenced by the observations that deficiency of folic acid impedes collagen formation, and folic acid deficiency, too, has the potential to impair adhesion of macrophages to collagen. Furthermore, M2 macrophages have been found to mediate collagen remodelling and degradation in a number of tissues [16–18,62–64]. These relationships illustrate the importance of some of the potential interactions between folic acid, M2-like macrophages, and molecules involved in structural formation and closure of the neural tube.

Discussion

A large body of superficial evidence appears to support a connection between forces which protect and polarize macrophages (or pre-macrophages) and forces which are already known to influence neural tube closure. In particular, the multidimensional role for folic acid, an important preventative agent for NTDs, on macrophage regulation - from facilitation of methylation, to interactions with folate receptors, to macrophage interactions with collagen - suggests a potential role for M2-like macrophages or pre-macrophages in neural tube closure which is both central and highly protected.

Similarly, the multifaceted protective roles for inositol related factors such as IP3 and S1P on macrophages suggest a potential key role for M2-like macrophages or pre-macrophages in a different molecular and physiological setting. After all, IP3 and S1P stimulate macrophage survival, chemotaxis, and polarization. Furthermore, IP3 stimulates macrophage migration via Rac1, via the same association that also governs neural tube closure. It is reasonable to postulate that some of the protective and regulatory processes known to apply to macrophages might also apply to embryonic pre-macrophages.

Though this paper has presented a number of intriguing associations, associations do not prove causality. It is possible that the macrophage-influencing properties of neurulation-influencing factors might be unrelated to neural tube closure. Nevertheless, given the contribution of macrophages to deposition, organization, and regulation of structural proteins necessary for neural tube closure, a potential causative connection between macrophages and closure of the neural tube seems likely.

A potential role for macrophages or pre-macrophages in neural tube closure could have important clinical implications. Folic acid exerts a preventative effect on some but not all NTDs, and NTD models also exist which are resistant to inositol and formate. Additional preventative therapies are needed to fill the gap. To this end, therapies which target macrophages of certain phenotypes could have utility in the modulation of risk for NTDs – especially in conditions known to be less sensitive to folic acid or other forms of prophylaxis.

A corollary of the M2 macrophage hypothesis for NTDs relates to support for a potential use for sildenafil in the context of gestational diabetes mellitus. Diabetes mellitus is a proinflammatory state, and evidence suggests that diabetes mellitus may decrease anti-inflammatory activity of macrophages or pre-macrophages, at least in part, through mechanisms related to nitric oxide excess. As mentioned previously, sildenafil is a phosphodiesterase inhibitor which stabilizes nitric oxide and which appears to prevent oxidation of nitric oxide to peroxynitrite. Under conditions of nitric oxide excess, nitric oxide-derived peroxynitrite decreases the sensitivity of the NMDA receptor. Therefore it is possible that sildenafil, by limiting peroxynitrite, might be capable of opposing diabetes-induced alterations in macrophage balance. Sildenafil has not been shown to present a significant risk to the developing fetus.

In addition to the above, an understanding of a role for macrophages in neural tube closure could help to identify additional risk factors or health promoters for the neural tube. For example, might intravenous immune globulin, which increases macrophage polarization to the M1 phenotype in vitro [65], introduce additional risks for NTDs? Might endurance exercise training or treatment with capsaicin, both of which promote macrophage polarization to the M2 phenotype in some models [66,67], reduce the risk for NTDs? Further research will be required to answer these and other questions.

Declaration of Competing Interest

None

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