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Archaeal Digoxin Mediated Model for Sarcoidosis

Sarcoidosis is a chronic, multisystem disorder of unknown cause characterised in affected organs by an accumulation of T-lymphocytes and mononuclear phagocytes, non-caseating epitheloid granulomas, and derangements of the normal tissue architecture. Sarcoidosis is characterized at the sites of the disease by exaggerated T-helper lymphocyte immune processes. All available evidence suggests that active sarcoidosis results from an exaggerated cellular immune response to a variety of antigens or self-antigens, in which the process of T-lymphocyte triggering, proliferation, and activation is skewed in the direction of helper-inducer T-lymphocyte processes. This result is an exaggerated helper-inducer T-cell response and thus the accumulation of large numbers of activated T-cell in the affected organs. Since the activated helper-inducer T-lymphocytes releases mediators that attract and activate mononuclear phagocytes, it is likely that the process of granuloma formation is a secondary phenomenon that is a consequence of the exaggerated helper-inducer T-cell process. The T-helper-inducer lymphocytes accumulate at the sites of disease, at least in part, because they proliferate in these sites at an exaggerated rate. This T-cell proliferation is maintained by the spontaneous release of IL-2, the T-cell growth factor, by activated T-helper-inducer cells in the local milieu. Geschwind has postulated a relationship between cerebral lateralization and immune function. For example, they observed a higher frequency of left handedness in patients with some immune disorders. There are no reports on the role of hemispheric dominance in the pathogenesis of sarcoidosis.

The isoprenoid pathway is a key regulatory pathway in the cell. It produces three key metabolites - digoxin, ubiquinone and dolichol important in cellular function. The endosymbiotic archaea synthesizes digoxin. Elevated levels of archaeal digoxin have been documented in immune mediated disease. Digoxin can also alter neurotransmitter transport. Alteration in monoamine neurotransmitters have been described in immune mediated disorders.

Alteration in protein processing has also been documented in immune mediated disorders. Dolichol is important in N-glycosylation of proteins. It was therefore considered pertinent to study the isoprenoid pathway in sarcoidosis. As hypothalamic archaeal digoxin can modulate synaptic transmission of multiple neurotransmitter systems, the pathway was also assessed in individuals with differing hemispheric dominance to find out the role of hemispheric dominance in the pathogenesis of sarcoidosis.

Results

(1) The activity of HMG CoA reductase and the concentration of digoxin and dolichol were increased in systemic sarcoidosis. The concentration of serum ubiquinone, the activity of erythrocyte membrane Na^{+}-K^{+} ATPase and serum magnesium were decreased in systemic sarcoidosis.

(2) The concentration of serum tryptophan, quinolinic acid and serotonin were increased in the plasma while that of tyrosine, dopamine and noradrenaline were decreased in systemic sarcoidosis.

(3) Nicotine and strychnine were detected in the plasma of patients with systemic sarcoidosis and were undetectable in control serum. Morphine was not detected in the plasma of systemic sarcoidosis.

(4) The concentration of total GAO increased in the serum of systemic sarcoidosis patients. The concentration of hyaluronic acid (HA), heparan sulphate (HS), heparin (H), dermatan sulphate (DS) and chondroitin sulphates (ChS) were increased in systemic sarcoidosis. The concentration of total hexose, fucose and sialic acid were increased in the glycoproteins of the serum in systemic sarcoidosis.

(5) The activity of GAG degrading enzymes beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase, cathepsin-D, were increased in

systemic sarcoidosis when compared to the controls. The activity of beta galactosidase, beta fucosidase and beta glucosidase increased in systemic sarcoidosis.

(6) The concentration of total GAG, hexose and fucose in the RBC membrane decreased significantly in systemic sarcoidosis. The concentration cholesterol increased and phospholipids decreased in the RBC membrane in systemic sarcoidosis and the cholesterol: phospholipid ratio in the RBC membrane increased significantly in systemic sarcoidosis.

(7) The activity of superoxide dismutase (SOD), catalase, glutathione reductase and glutathione peroxidase in the erythrocytes decreased significantly in systemic sarcoidosis. In sarcoidosis the concentration of MDA, hydroperoxides, conjugated dienes and NO increased significantly. The concentration of reduced glutathione decreased in systemic sarcoidosis.

(8) The results showed that HMG CoA reductase activity, serum digoxin and dolichol were increased and ubiquinone reduced in left handed / right hemispheric dominant individuals. The results also showed that HMG CoA reductase activity, serum digoxin and dolichol were decreased and ubiquinone increased in right handed / left hemispheric dominant individuals. The results showed that the concentration of tryptophan, quinolinic acid serotonin, strychnine and nicotine was found to be higher in the plasma of left handed / right hemispheric dominant individuals while that of tyrosine, dopamine, morphine and norepinephrine was lower. The results also showed that the concentration of tryptophan, quinolinic acid serotonin, strychnine and nicotine was found to be lower in the plasma of right handed / left hemispheric dominant individuals while that of tyrosine, dopamine, morphine and norepinephrine was higher.

Discussion

Archaeal Digoxin and Membrane Na^+-K^+ ATPase Inhibition in Relation to Sarcoidosis

The archaeaon steroidelle DXP pathway and the upregulated pentose phosphate pathway contribute to digoxin synthesis. The results showed that HMG CoA reductase activity, serum digoxin and doliohol were increased in systemic sarcoidosis while serum ubiquinone was reduced. Previous studies in this laboratory have demonstrated incorporation of ^{14}C-acetate into digoxin in rat brain indicating that acetyl CoA is the precursor for digoxin biosynthesis in mammals also. The elevated HMG CoA reductase activity correlates well with elevated digoxin levels and reduced RBC membrane Na^+-K^+ ATPase activity. The increase in endogenous digoxin, a potent inhibitor of membrane Na^+-K^+ ATPase, can decrease this enzyme activity. The inhibition of Na^+-K^+ ATPase by digoxin is known to cause an increase in intracellular calcium resulting from increased Na^+-Ca^{++} exchange, increased entry of calcium via the voltage gated calcium channel and increased release of calcium from intracellular endoplasmic reticulum calcium stores. This increase in intracellular calcium by displacing magnesium from its binding sites, causes a decrease in the functional availability of magnesium. This decrease in the availability of magnesium can cause decreased mitochondrial ATP formation which along with low magnesium can cause further inhibition of Na^+-K^+ ATPase, since ATP-magnesium complex is the actual substrate for this reaction. Cytosolic free calcium is normally buffered by two mechanisms, ATP dependent calcium extrusion from cell and ATP dependent sequestration of calcium within the endoplasmic reticulum. The magnesium related mitochondrial dysfunction results in defective calcium extrusion from the cell. There is thus a progressive inhibition of Na^+-K^+ ATPase activity first triggered by digoxin. Low intracellular magnesium and high intracellular calcium consequent to Na^+-K^+

ATPase inhibition appear to be crucial to the pathogenesis of systemic sarcoidosis. Serum magnesium was found to be reduced in systemic sarcoidosis.

Archaeal Digoxin and Immune Activation - Relation to Sarcoidosis

The archaeaon fructosoid contributes to fructolysis and immune activation. Fructose can contribute to induction of NFKB and immune activation. The archaeaon steroidelle synthesized digoxin induces NFKB producing immune activation. Increased intracellular calcium activates calcium dependent calcineurin signal transduction pathway which can produce T-cell and macrophage activation with secretion of interleukin-2, 3, 4, 5, 6, 8 and TNF alpha. This can also explain the immune activation in systemic sarcoidosis. Sarcoidosis is characterised by an exaggerated T-helper-inducer response. There is an accumulation of large numbers of activated T-cell in the affected organs. Since the activated helper-inducer T-lymphocytes releases mediators that attract and activate mononuclear phagocytes, it is likely that the process of granuloma formation is a secondary phenomenon that is a consequence of the exaggerated helper-inducer T-cell processes. The T-helper-inducer lymphocytes accumulate at the Sites of disease, at least in part, because they proliferate in these sites at an exaggerated rate. This T-cell proliferation is maintained by the spontaneous release of IL-2, the T-cell growth factor, by activated T-helper-inducer cells in the local milieu. The increase in intracellular calcium consequent to membrane Na^+-K^+ ATPase inhibition can increase the function of IL-2 whose receptor is a G-protein coupled. In addition to driving other T-helper - inducer cells in the affected organs to proliferate, the T-helper-inducer cells at the sites of disease are activated and release mediators like TNF alpha that both recruit and activate mononuclear phagocytes. Membrane Na^+-K^+ ATPase inhibition can produce immune activation and is reported to increase CD_4 / CD_8 ratios as exemplified by the action of lithium.

Archaeal Digoxin and Regulation of Neurotransmitter Synthesis and Function in Relation to Sarcoidosis

The archaeaon neurotransminoid shikimic acid pathway contributes to tryptophan and tyrosine synthesis and catabolism generating neurotransmitters and neuroactive alkaloids. Digoxin, apart from affecting cation transport is also reported to influence the transport of various metabolites across cellular membranes, including amino acids and various neurotransmitters. The results showed that the concentration of tryptophan, quinolinic acid and serotonin were found to be higher in the plasma of patients with sarcoidosis while that of tyrosine, dopamine and norepinephrine were lower. Thus there is an increase in tryptophan and its catabolites and a reduction in tyrosine and its catabolites in the serum of systemic sarcoidosis patients. This could be due to the fact that digoxin can regulate neutral amino acid transport system with preferential promotion of tryptophan transport over tyrosine. The decrease in membrane Na^+-K^+ ATPase activity in systemic sarcoidosis could be due to the fact that the hyperpolarising neurotransmitters (dopamine, noradrenaline and morphine) are reduced and the depolarising neuroactive compounds (serotonin, quinolinic acid, strychnine and nicotine) are increased. Quinolinic acid has been implicated in immune activation in other immune diseases and could contribute to the same in systemic sarcoidosis. Serotonin, dopamine and noradrenaline receptors have been demonstrated in the lymphocytes. It has been reported that during immune activation serotonin is increased with the corresponding reduction in dopamine and noradrenaline in the brainstem monoaminergic nuclei. Thus elevated serotonin and reduced noradrenaline and dopamine can contribute to the immune activation in systemic sarcoidosis. We had already shown the presence of endogenous morphine in the brain of rats loaded with tyrosine and endogenous strychnine and nicotine in the brain of rats loaded with tryptophan. Serum of patients with systemic sarcoidosis showed the presence of strychnine and nicotine but morphine was absent. The

absence of morphine in patients with systemic sarcoidosis is also significant. Morphine can inhibit the neutrophilic inflammatory response and the absence of morphine could contribute to an exaggeration of this response. Gamma interferons released by an activated T-cell can activate and recruit mononuclear phagocytes in sarcoidosis. Gamma interferons act by inducing the enzyme indoleamine 2, 3-dioxygenase and promoting tryptophan catabolism. The increased levels of tryptophan and its catabolitic products consequent to elevated digoxin levels can promote gamma interferon action.

Archaeal Digoxin and Regulation of Golgi Body / Lysosomal Function in Relation to Sarcoidosis

The archaeaon glycosaminoglycoid and fructosoid contributes to glycoconjugate synthesis and catabolism by the process of fructolysis. The low magnesium levels consequent to membrane Na^{+}-K^{+} ATPase inhibition can affect the metabolism of glycosaminoglycans, glycoproteins and glycolipids. The elevation in the level of dolichol consequent to its increased synthesis, may suggest its increased availability of N-glycosylation of proteins. Decrease in intracellular magnesium can produce upregulation of collagen and elastin biosynthesis and produce replacement fibrosis. In magnesium deficiency the glycolysis, citric acid cycle and oxidative phosphorylation are blocked and more glucose 6-phosphate is channelled for the synthesis of glycosaminoglycans (GAG). The results showed an increase in the concentration of serum total GAG, and carbohydrate components of glycoproteins (hexose, fucose and sialic acid) in systemic sarcoidosis. The increase in the carbohydrate components of serum glycoproteins - total hexose, fucose and sialic acid was not to the same extent in systemic sarcoidosis suggesting qualitative change in glycoprotein structure. In systemic sarcoidosis the percentage change in total hexose, fucose and sialic acid when compared to control is 54.3%, 20% and 33% respectively. The

concentration of hyaluronic acid, heparan sulphate, heparin, dermatan sulphate and chondroitin sulphates were increased in the serum of systemic sarcoidosis patients. The activity of GAG degrading enzymes (beta glucuronidase, beta N-acetyl hexosaminidase, hyaluronidase and cathepsin-D) were increased in the serum of systemic sarcoidosis patients. The activity of glycohydrolases - beta galactosidase, beta fucosidase and beta glucosidase was increased in the serum of systemic sarcoidosis patients. The increase in the activity of glycohydrolases and GAG degrading enzymes could be due to reduced lysosomal stability and consequent leakage of lysosomal enzymes into the serum. The increase in the concentration of carbohydrate components of glycoproteins and GAG inspite of increased activity of many glycohydrolases may be due to their possible resistance to cleavage by glycohydrolases consequent to qualitative change in their Structure. A number of fucose and sialic acid containing natural ligands are involved in trafficking of leukocytes and adhesion of the lymphocyte producing leukocyte trafficking and extravasation in to the perivascular space. The increase in sialoligands and fucoligands could produce the same in systemic sarcoidosis. The upregulated glycoproteins and glycosaminoglycans synthesis can contribute to the replacement fibrosis in systemic sarcoidosis.

The protein processing defect can result in defective glycosylation of endogenous lung glycoprotein antigens and exogenous viral glycoprotein antigens with consequent defective formation of MHC-antigen complex. The MHC linked peptide transporter, a P-glycoprotein which transports MHC-antigen complex to the antigen presenting cell surface, has an ATP binding site which is dysfunctional in the presence of magnesium deficiency. This results in defective transport of MHC class-1 lung / tissue glycoprotein antigen complex to the antigen presenting cell surface for recognition by CD_4 or CD_8 cell. Defective presentation of endogenous lung / tissue glycoprotein antigen can explain the immune dysregulation in systemic sarcoidosis. This can

contribute towards the autoimmunity in systemic sarcoidosis. Thus a protein processing dysfunction can lead to the generation of self antigens in various tissues contributing to autoimmunity. Defective exogenous glycoprotein antigen presentation consequent to a defective MHC antigen presenting pathway can contribute to cutaneous anergy in systemic sarcoidosis.

There are other factors that contribute towards replacement fibrosis in systemic sarcoidosis. Several products from macrophages can participate in these steps. PDGF-B is a chemoattractant for mesenchymal cells and a stimulus for fibroblasts to change from resting cells to cells entering GI. Although PDGF-B is not produced by normal macrophages, macrophages obtained from patients with systemic sarcoidosis make it abundantly. This is correlated with c-sis, a proto-oncogene the codes for the beta chain of PDGF-B which is increased in sarcoid granuloma derived tuacrophages. Gamma interferon upregulates this gene activation. PDGF receptor is a G-protein coupled receptor. The increase in intracellular calcium consequent to membrane Na^{+}-K^{+} ATPase inhibition can upregulate its activity. Increased intracellular calcium activates phospholipase C beta which results in increased production of diacyglycerol (DAG) with resultant activation of protein kinase C. The protein kinase C (PKC) activates the MAP kinase cascade resulting in cellular proliferation. This can activate the c-sis proto-oncogene the codes for the beta chain of PDGF-B. Increased levels of PDGF can contribute to fibroblast proliferation in sarcoidosis.

Archaeal Digoxin and Alteration in Membrane Structure and Membrane Formation in Relation to Sarcoidosis

The archaeaon steroidelle, glycosaminoglycoid and fructosoid contribute to cell membrane formation synthesizing cholesterol by the DXP pathway and glycosaminoglycans by fructolysis. The alteration in the isoprenoid pathway specifically, cholesterol as well as changes in glycoproteins and GAG can affect

cellular membranes. The upregulation of the isoprenoid pathway can lead to increased cholesterol synthesis and magnesium deficiency can inhibit phospholipid synthesis. Phospholipid degradation is increased owing to increase in intracellular calcium activating phospholipase A_2 and D. The membrane composition was assessed by, RBC membrane cholesterol: phospholipid ratio, carbohydrate residues of glycoproteins and total glycosaminoglycans. The cholesterol: phospholipid ratio of the RBC membrane was increased in systemic sarcoidosis. The concentration of total GAG, hexose and fucose of glycoprotein and cholesterol decreased in the RBC membrane and increased in the serum suggesting their reduced incorporation in to the membrane and defective membrane formation. The glycoproteins, GAG and glycolipids of the cellular membrane are formed in the endoplasmic reticulum, which is then budded off as a vesicle which fuses with the golgi complex. The glycoconjugates are then transported via the golgi channel and the golgi vesicle fuses with the cell membrane. This trafficking depends upon GTPases and lipid kinases which are crucially dependent on magnesium and are defective in magnesium deficiency. The change in membrane structure produced by alteration in glycoconjugates and cholesterol: phospholipid ratio can produce changes in the conformation of Na^+-K^+ ATPase resulting in further membrane Na^+-K^+ ATPase inhibition.

Archaeal Digoxin and Mitochondrial Dysfunction in Relation to Sarcoidosis

The archaeaon vitaminocyte contributes to the synthesis of ubiquinone and mitochondrial electron transport chain function. The mitochondrial function related free radical generation is regulated by the archaeaon vitaminocyte synthesized tocopherol and ascorbic acid. The concentration of ubiquinone decreased significantly in systemic sarcoidosis which may be the result of low tyrosine levels, reported in systemic sarcoidosis consequent to digoxin's effect

in preferentially promoting tryptophan transport over tyrosine. The aromatic ring portion of ubiquinone is derived from the tyrosine. Ubiquinone is important and contributes to free radical scavenging. The increase in intracellular calcium can open the mitochondrial PT pore causing a collapse of the hydrogen gradient across the inner membrane and uncoupling of the respiratory chain. Intracellular magnesium, deficiency can lead to a defect in the function of ATP synthase. All this leads to a defect in mitochondrial oxidative phosphorylation, incomplete reduction of oxygen and generation of superoxide ion which produces lipid peroxidation. Ubiquinone deficiency also leads to reduced free radical scavenging. The increase in intracellular calcium may lead to increased generation of NO by inducing the enzyme nitric oxide synthase which combines with superoxide radical to form peroxynitrite. Increased calcium also can activate phospholipase A_2 resulting in increased generation of arachidonic acid which can undergo increased lipid peroxidation. Increased generation of free radicals like the superoxide ion, and hydroxyl radical can produce lipid peroxidation and cell membrane damage which can further inactivate Na^+-K^+ ATPase triggering the cycle of free radical generation again. The free radicals and scavenging enzymes were estimated in sarcoidosis. There was an increase in lipid peroxidation as evidenced from the increase in the concentration of MDA, conjugated dienes, hydroperoxides and NO with decreased antoxidant protection as indicated by a decrease in ubiquinone and reduced glutathione in systemic sarcoidosis. The activity of enzymes involved in free radical scavenging like superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and catalase is decreased in systemic sarcoidosis suggesting reduced free radical scavenging. The peroxisomal membrane is defective owing to membrane Na^+-K^+ ATPase inhibition related defect in membrane formation and leads to reduced catalase activity. Glutathione synthetase and glutathione peroxidase need magnesium for their activity. The

glutathione system of free radical scavenging is defective in the presence of membrane Na^+-K^+ ATPase inhibition. Superoxide dismutase exists in a mitochondrial and cytoplasmic form. Opening of the mitochondrial PT pore produces hyperosmolality and matrix expansion rupturing the outer membrane producing loss of the mitochondrial dismutase and a decrease in its activity. The reduction in catalase, superoxide dismutase (SOD), glutathione peroxidase and glutathione reductase suggests reduced free radical protection. Mitochondrial dysfunction related free radical generation has been implicated in the pathogenesis of the systemic sarcoidosis. Free radicals are involved in immune activation.

Archaeal Digoxin and Hemispheric Dominance in Relation to Sarcoidosis

The archaeaon related organelle-steroidelle, neurotransminoid and vitaminocyte contribute to hemispheric dominance. The biochemical patterns in sarcoidosis correlated with right hemispheric chemical dominance. In left handed / right hemispheric dominant individuals there was a derangement of the isoprenoid pathway. They had an upregulated HMG CoA reductase activity with increased digoxin and dolichol levels and reduced ubiquinone levels. The RBC membrane Na^+-K^+ ATPase activity was reduced and serum magnesium depleted. The left handed / right hemispheric dominant individuals had increased levels of tryptophan, serotonin, quinolinic acid, strychnine and nicotine while the levels of tyrosine, dopamine, noradrenaline and morphine were lower. Thus an upregulated isoprenoid pathway, increased level of tryptophan and its catabolites decreased levels of tyrosine and its catabolites and hyperdigoxinemia is suggestive of right hemispheric dominance. The opposite patterns occur in left hemispheric dominance. Systemic sarcoidosis thus occurs in right hemisphere dominant individuals and is a reflection of altered brain

function occurring in right hemisphere dominant individuals. Hemispheric dominance plays an important role in modulating immune function.

References

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