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Endosymbiotic Actinidic Archaea and Viroids-Role in Immune Regulation

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Abstract

Objective: A hypothesis regarding the role of endosymbiotic actinidic archaea and viroids in immune regulation is put forward. Endogenous digoxin has been related to the pathogenesis of multiple sclerosis and other autoimmune diseases like systemic lupus erythematosis and rheumatoid arthritis. The possibility of endogenous digoxin synthesis by actinidic archaea with a mevalonate pathway and cholesterol catabolism was considered. The role of archaeal derived viroids in immune regulation was also evaluated.

Methods: 10 cases each of multiple sclerosis and other autoimmune diseases like systemic lupus erythematosis and rheumatoid arthritis before starting treatment and 10 age and sex matched healthy controls from general population were chosen for the study. Cholesterol substrate was added to the plasma of the patients and the generation of cytochrome F420, free RNA, free DNA, polycyclic aromatic hydrocarbon, hydrogen peroxide, serotonin, pyruvate, ammonia, glutamate, cytochrome C, hexokinase, ATP synthase, HMG CoA reductase, digoxin and bile acids were studied. The changes with the addition of antibiotics and rutile to the patient's plasma were also studied. The statistical analysis was done by ANOVA.

Results: The parameters mentioned above were increased the patient's plasma with addition of cholesterol substrate. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutile increased their levels.

Conclusions: An actinide dependent shadow biosphere of archaea and viroids is described in multiple sclerosis and other autoimmune diseases like systemic lupus erythematosis and rheumatoid arthritis contributing to their pathogenesis. The actinidic archaea and viroids play a role in immune regulation.

Key words: Archaea; Viroids; Cholesterol; Actinide; Multiple sclerosis; Systemic lupus erythematosis; Rheumatoid arthritis; Immune regulation

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INTRODUCTION

A hypothesis regarding the role of endosymbiotic actinidic archaea and viroids in immune regulation is put forward. Endomyocardial fibrosis (EMF) along with the root wilt disease of coconut is endemic to Kerala with its radioactive actinide beach sands. Actinides like rutile producing intracellular magnesium deficiency due to rutile-magnesium exchange sites in the cell membrane has been implicated in the etiology of EMF[1]. Endogenous digoxin, a steroidal glycoside which functions as a membrane sodium-potassium ATPase inhibitor has also been related to its etiology due to the intracellular magnesium deficiency it produces[2]. Organisms like phytoplasmas and viroids have also been demonstrated to play a role in the etiology of these diseases[3, 4]. Endogenous digoxin has been related to the pathogenesis of multiple sclerosis and other autoimmune diseases like systemic lupus erythematosis and rheumatoid arthritis[2]. The possibility of endogenous digoxin synthesis by actinide based primitive organism like archaea with a mevalonate pathway and cholesterol catabolism was considered[5-8]. An actinide dependent shadow biosphere of archaea and viroids in the above mentioned disease states

is described[6]. The role of actinidic archaea and viroids in immune regulation is elucidated in this paper.

MATERIALS AND METHODS

Informed consent of the subjects and the approval of the ethics committee were obtained for the study. The following groups were included in the study:- multiple sclerosis, systemic lupus erythematosis and rheumatoid arthritis. There were 10 patients in each group and each patient had an age and sex matched healthy control selected randomly from the general population. The blood samples were drawn in the fasting state before treatment was initiated. Plasma from fasting heparinised blood was used and the experimental protocol was as follows (I) Plasma+phosphate buffered saline, (II) same as I+cholesterol substrate, (III) same as II+rutile 0.1 mg/ml, (IV) same as II+ciprofloxacine and doxycycline each in a concentration of 1 mg/ml. Cholesterol substrate was prepared as described by Richmond[9]. Aliquots were withdrawn at zero time immediately after mixing and after incubation at 37°C for 1 hour. The following estimations were carried out:- Cytochrome F420, free RNA, free DNA, polycyclic aromatic hydrocarbon, hydrogen peroxide, pyruvate, glutamate, hexokinase, ATP synthase, HMGCoA reductase, digoxin and bile acids[10,11,12,13]. Cytochrome F420 was estimated flourimetrically (excitation wavelength 420 nm and emission wavelength 520 nm). Polycyclic aromatic hydrocarbon was estimated by measuring hydrogen peroxide liberated by using glucose reagent. The statistical analysis was done by ANOVA.

RESULTS

The parameters checked as indicated above were:- cytochrome F420, free RNA, free DNA, muramic acid, polycyclic aromatic hydrocarbon, hydrogen peroxide, serotonin, pyruvate, ammonia, glutamate, cytochrome C, hexokinase, ATP synthase, HMG CoA reductase, digoxin and bile acids. Plasma of control subjects showed increased levels of the above mentioned parameters with after incubation for 1 hour and addition of cholesterol substrate resulted in still further significant increase in these parameters. The plasma of patients showed similar results but the extent of increase was more. The addition of antibiotics to the control plasma caused a decrease in all the parameters while addition of rutile increased their levels. The addition of antibiotics to the patient's plasma caused a decrease in all the parameters while addition of rutile increased their levels but the extent of change was more in patient's sera as compared to controls. The results are expressed in tables 1-7 as percentage change in the parameters after 1 hour incubation as compared to the values at zero time.

Table 1
Effect of Rutile and Antibiotics on ATP Synthase and Cytochrome F420

	ATP synthase % (Increase with Rutile)		ATP synthase % (Decrease with Doxy)		CYT F420 % (Increase with Rutile)		CYT F420 % (Decrease with Doxy)	
Group	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.40	0.11	18.78	0.11	4.48	0.15	18.24	0.66
MS	23.52	1.76	67.05	3.00	22.12	1.81	61.33	9.82
SLE	23.04	1.66	66.13	3.49	21.65	2.25	61.19	7.90
RA	23.29	1.98	67.46	3.96	22.22	1.76	53.92	7.12
F value	449.503		673.081		306.749		130.054	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 2
Effect of Rutile and Antibiotics on Free DNA and RNA

	DNA % change (Increase with Rutile)		DNA % change (Decrease with Doxy)		RNA % change (Increase with Rutile)		RNA % change (Decrease with Doxy)	
Group	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.37	0.15	18.39	0.38	4.37	0.13	18.38	0.48
MS	22.62	1.38	63.82	5.53	23.29	1.98	67.46	3.96
SLE	21.94	2.03	64.29	5.35	23.75	1.81	66.49	4.11
RA	21.93	2.29	63.70	5.63	23.52	1.76	67.05	3.00
F value	337.577		356.621		427.828		654.453	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 3
Effect of Rutile and Antibiotics on HMG CoA Reductase and PAH

	HMG CoA R % change (Increase with Rutile)		HMG CoA R % change (Decrease with Doxy)		PAH % change (Increase with Rutile)		PAH % change (Decrease with Doxy)	
Group	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.30	0.20	18.35	0.35	4.45	0.14	18.25	0.72
MS	23.14	1.85	59.76	4.82	22.83	1.78	59.84	7.62
SLE	22.64	1.99	64.93	7.62	22.91	2.13	61.96	6.10
RA	23.42	1.76	65.71	3.28	22.26	1.90	61.80	6.12
F value	319.332		199.553		391.318		257.996	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 4
Effect of Rutile and Antibiotics on Digoxin and Bile Acids

	Digoxin (ng/ml) (Increase with Rutile)		Digoxin (ng/ml) (Decrease with Doxy+Cipro)		Bile acids % change (Increase with Rutile)		Bile acids % change (Decrease with Doxy)	
Group	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normalt	0.11	0.00	0.054	0.003	4.29	0.18	18.15	0.58
MS	0.52	0.03	0.214	0.032	21.95	2.11	65.46	5.79
SLE	0.54	0.05	0.227	0.036	22.88	1.99	63.92	6.74
RA	0.50	0.06	0.228	0.027	23.35	2.05	67.25	4.72
F value	135.116		71.706		290.441		203.651	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 5
Effect of Rutile and Antibiotics on Pyruvate and Hexokinase

	Pyruvate % change (Increase with Rutile)		Pyruvate % change (Decrease with Doxy)		Hexokinase % change (Increase with Rutile)		Hexokinase % change (Decrease with Doxy)	
Group	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.34	0.21	18.43	0.82	4.21	0.16	18.56	0.76
MS	21.59	1.23	60.28	9.22	22.81	1.91	63.47	5.81
SLE	20.01	1.73	58.08	8.16	21.84	2.08	63.56	5.23
RA	20.08	1.84	61.70	7.17	22.65	1.99	64.05	4.89
F value	321.255		115.242		292.065		317.966	
P value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 6
Effect of Rutile and Antibiotics on Hydrogen Peroxide and Glutamate

	H_2O_2 % (Increase with Rutile)		H_2O_2 % (Decrease with Doxy)		Glutamate% change (Increase with Rutile)		Glutamate% change (Decrease with Doxy+Cipro)	
Group	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**	**Mean**	**± SD**
Normal	4.43	0.19	18.13	0.63	4.34	0.21	18.43	0.82
MS	21.14	1.20	60.53	4.70	21.59	1.23	60.28	9.22
SLE	23.71	1.64	59.51	6.63	21.19	1.61	58.57	7.47
RA	23.03	1.66	61.91	6.31	22.29	2.05	62.37	5.05
F value P value	380.721 < 0.001		171.228 < 0.001		F value 321.255 P value < 0.001		F value 115.242 P value < 0.001	

Abbreviations:
MS: Multiple sclerosis
SLE: Systemic lupus erythematosis
RA: Rheumatoid arthritis

DISCUSSION

There was increase in cytochrome F420 indicating archaeal growth in multiple sclerosis , systemic lupus erythematosis and rheumatoid arthritis. The archaea can synthesise and use cholesterol as a carbon and energy source[14,15]. The archaeal origin of the enzyme activities was indicated by antibiotic induced suppression. The study indicates the presence of actinide based archaea with an alternate actinide based enzymes or metalloenzymes in the system as indicated by rutile induced increase in enzyme activities[16]. There was also an increase in archaeal HMG CoA reductase activity indicating increased cholesterol synthesis by the archaeal mevalonate pathway. The archaeal beta hydroxyl steroid dehydrogenase activity indicating digoxin synthesis and archaeal cholesterol hydroxylase activity indicating bile acid synthesis were increased[7]. The archaeal cholesterol oxidase activity was increased resulting in generation of pyruvate and hydrogen peroxide[15]. The pyruvate gets converted to glutamate and ammonia by the GABA shunt pathway. The archaeal aromatization of cholesterol generating PAH was also detected[17]. The archaeal glycolytic hexokinase activity and archaeal extracellular ATP synthase activity were increased. The archaea can undergo magnetite and calcium carbonate mineralization and can exist as calcified nanoforms[18].

There was an increase in free RNA indicating self replicating RNA viroids and free DNA indicating generation of viroid complementary DNA strands by archaeal reverse transcriptase activity. The actinides modulate RNA folding and catalyse its ribozymal action. Digoxin can cut and paste the viroidal strands by modulating RNA splicing generating RNA viroidal diversity. The viroids are evolutionarily escaped archaeal group I introns which have retrotransposition and self splicing qualities[19]. Archaeal pyruvate can produce histone deacetylase inhibition resulting in endogenous retroviral (HERV) reverse transcriptase and integrase expression. This can integrate the RNA viroidal complementary DNA into the noncoding region of eukaryotic non coding DNA using HERV integrase as has been described for borna and ebola viruses[20-22]. The archaea genome can also get integrated into human genome using integrase as has been described for trypanosomes[21]. The integrated viroids and archaea can undergo vertical transmission and can exist as genomic parasites[20,21]. The viroidal complementary DNA can function as jumping genes producing a dynamic genome important in HLA gene expression. This modulation of HLA gene expression by viroidal complementary DNA can result in autoimmune diseases. The RNA viroids can regulate mrna function by RNA interference[19]. The phenomena of RNA interference can modulate T cell and B cell function and euchromatin/ heterochromatin expression. RNA viroidal mRNA interference plays a role in the pathogenesis of autoimmune diseases like systemic lupus erythematosis, rheumatoid arthritis and multiple sclerosis. The integration of nanoarchaea and viroids in to the eukaryotic and human genome produces a chimera which can multiply producing biofilm like multicellular structures having a mixed archaeal, viroidal and eukaryotic characters which is a regression from the multicellular eukaryotic tissue This results in a new immune and tissue phenotype leading to human diseases like systemic lupus erythematosis, rheumatoid arthritis and multiple sclerosis. The microchimeras formed can lead to autoantigens and autoimmune diseases[23-37].

Archaea and RNA viroid can bind the TLR receptor induce NFKB producing immune activation and cytokine TNF alpha secretion. The archaeal DXP and mevalonate pathway metabolites can bind γδ TCR and digoxin induced calcium signaling can activate NFKB producing chronic immune activation[2, 38]. The archaea and viroid induced chronic immune activation and generation of superantigens can lead on to autoimmune disease. The higher degree of integration of the archaea into the genome produces increased digoxin synthesis producing right hemispheric dominance and lesser degree producing left hemispheric dominance[2]. Right hemispheric dominance can lead to autoimmune diseases.

Archaea, viroids and digoxin can induce the host AKT PI3K, AMPK, HIF alpha and NFKB producing the Warburg metabolic phenotype[39]. The increased glycolytic hexokinase activity, decrease in blood ATP, leakage of cytochrome C, increase in serum pyruvate and decrease in acetyl CoA indicates the generation of the Warburg phenotype. There is induction of glycolysis, inhibition of PDH activity and mitochondrial dysfunction resulting in inefficient energetics. The lymphocytes depend on glycolysis for their energy needs. The increased glycolysis induced by the Warburg phenotype leads to immune activation. Lactic acid generated by increased glycolysis leads to immune stimulation. Cholesterol oxidase activity, increased glycolysis related NADPH oxidase activity and mitochondrial dysfunction generates free radicals important in the pathogenesis of these disease states. The accumulated pyruvate enters the GABA shunt pathway and is converted to citrate which is acted upon by citrate lyase and converted to acetyl CoA, used for cholesterol synthesis[39]. The pyruvate can be converted to glutamate and ammonia which is oxidised by archaea for energy needs. The increased cholesterol substrate leads to increased archaeal growth and digoxin synthesis leading to metabolic channeling to the mevalonate pathway.

Hyperdigoxinemia is important in the pathogenesis of autoimmune disease. Digoxin can increase lymphocytic intracellular calcium which leads on to induction of NFKB and immune activation[2]. The archaeal cholesterol catabolism can deplete the lymphocytic cell membranes of cholesterol resulting in alteration of lymphocytic cell

membrane microdomains related receptors producing immune activation. Endosymbiotic actinidic archaea and viroids can play a pivotal role in immune regulation.

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