SUMMARY OF SIGNIFICANT PAPERS

1)PK Sahoo, JJ Babugedam, AK Satpathy, MC Mohanty, **BK Das**, N Mishra, B Ravindran **(2002**). **Bancroftian filariasis: a 13 year follow up study of asymptomatic microfilariae carriers and endemic normal in Orissa, India. Parasitology, 124: 191-201**

This is a seminal paper that observed the natural history of human lymphatic filarial infections leading to development of disease. It has been a subject of intense debate regarding the development of disease when infected with filariasis.The models proposed so far had largely been based on cross-sectional data on microfilariae (Mf) and disease prevalence in filariasis endemic areas. In an attempt to study the parasitological and clinical consequences of filarial infection in a Bancroftian filarial endemic area of Odisha, a cohort of 59 asymptomatic Mf carriers (AS) and 187 asymptomatic and amicrofilaraemic subjects or `endemic normals' (`EN'), were followed-up, and a fraction (73% and 46% respectively) re-examined after 13 years to monitor (a) Mf prevalence, (b) Mf density, (c) circulating filarial antigen(CFA) and (d) chronic disease manifestations. Both Mf prevalence and density decreased progressively in the cohort of Mf carriers over a period of 13 yrs. Only 37% of them continued to be microfilaraemic and the Mf density in these subjects was only 10% of the original level. However, loss of circulating Mf in this cohort did not result in loss of CFA and 95% remained CFA positive regardless of Mf status**.** Only 5.7% of male Mf carriers, who were not treated with DEC, developed hydrocoele after 13 years. These results revealed that in Mf carriers adult filarial worms persist for several years and that loss of circulating Mf with or without chemotherapy with DEC (single 12-day course) did not influence adult worm survival. These findings have helped in the understanding the ` static ' and `dynamic'models of disease pathogenesis describing the relationship between infection and disease in human filariasis.

This is one of the longest longitudinal studies tracing the natural history of Bancroftian filarial infection. It demonstrated that most infected subjects do not develop disease. Adult worms survive for a long time. It has a symbiotic relationship with the host, and its survival is based on immune evasion through multiple pathways which could be of great importance in suppressing autoimmune diseases.

2) AK Panda, **BKDas, (2014). Absence of filarial infection in patients of systemic lupus erythematosus (SLE) in filarial endemic area: a possible protective role. Lupus. 23,1553-4**

Systemic lupus erythematosus (SLE) is a chronic autoimmune disorder characterized by dysregulated autoantibody production, deposition of immune complex and failure of various organ systems. It primarily affects young women of child bearing age leading to devastating consequences. The etiology of SLE remains unknown however, genetic and environmental factors have been attributed to development of this disease. The environmental factors are UV light, viral infection, hormones and toxins. Nematode infections have been inversely associated with autoimmune disorders. Higher prevalence of autoimmune diseases in developed countries and lower in tropical countries are believed to be associated with nematode loads in the respective populations which is the basis of Hygiene Hypothesis. However, this hypothesis has never been studied in the context of the autoimmune diseases like SLE or

Rheumatoid arthritis. The cause and effect relationship between nematode infection and autoimmune disorders is not known. We believed, based on our earlier observations, that filarial infection may have some protective role in disease modulation in SLE. Females patients with SLE (n¼319), residing in lymphatic filarial endemic areas were enrolled. Also included were 263 healthy female healthy controls from a similar geographical area with no history of autoimmune disorder. 42% of healthy controls had filarial infection, which corroborated with our earlier observations. Interestingly, out of the 319 SLE patients, none showed CFA positivity although they lived in filarial endemic areas. Although this evidence had been demonstrated in the experimental model of collagen-induced arthritis (CIA) and in human subjects with rheumatoid arthritis by our group, this is the first report demonstrating an absence of filarial infection in patients with SLE. The precise mechanism(s) relating to the protective role of filarial infection in autoimmune disorders in human is not known. However, in the context of Collagen Induced Arthritis, reports have demonstrated that ES-62, the major excretory protein of rodent filarial nematode plays a protective role by suppressing IL-17 pathway.

3) Panda AK, **BKDas (2017).**Diminished IL17A levels may protect filarial infected subjects from development of rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE**). Lupus**.26(4):348-354

We had demonstrated earlier the absence of filarial infection in patients with RA and SLE .We believed that the Nematode infections which have been observed to have an inverse correlation with autoimmune disorders may have an important role in modulation of disease pathogenesis.. The mechanism(s) by which filarial-infected individuals are protected against the development of RA or SLE are unknown. In mice CIA, an experimental model for RA, ES-62, an execretory product of rodent filarial nematode, has been shown to improve arthritis through suppression of the IL-17 pathway. A total of 160 individuals, 40 each of endemic normal, filarial-infected cases, SLE and RA patients, from filarial-endemic areas, were enrolled in the study. Plasma levels of IL17-A, IFN-a and TNF-a were quantified by enzyme-linked immunosorbent assay (ELISA).RA and SLE patients displayed significantly higher plasma IL-17A, IFN-a and TNF-a levels compared to endemic normal and filaria infected individuals. Furthermore, IL-17A levels were significantly low in subjects with filarial infection compared to endemic controls. Interestingly, plasma IL-17A levels correlated inversely with circulating filarial antigen (CFA). Filarial infection was associated with low plasma IL-17A levels, a mechanism by which it possibly protects individuals in filarial-endemic areas from the development of autoimmune disorders like RA and SLE.

This is a seminal work which provides insight into the role of nematode infection on human autoimmune disorders. Understanding the nuances of the worm products has immense potential for generating synthetic analogues similar to ES 62 glycoprotein. Interestingly synthetic analogues of ES 62 have been tested in animal models of SLE with gratifying results.

4) M Mandal, R Tripathy, AK Panda, SS Pattanaik, S Dakua, AK Pradhan, S Chakraborty, B Ravindran, **BK Das, (2014**).Vitamin D levels in Indian systemic lupus erythematosus patients: association with disease activity index and interferon alpha. Arthritis Research and Therapy, 16(1):R49

The pathogenesis of SLE depends on genetic susceptibility and environmental factors. While viral infections trigger disease we demonstrated that nematode infection can be protective. Among the environmental factors, deficiency/insuffience of Vit D has been a subject of great debate as a possible trigger for autoimmune diseases. Low levels of vitamin D have been associated with several autoimmune disorders including multiple sclerosis, rheumatoid arthritis, type 1 diabetes and systemic lupus erythematosus (SLE). The major source of vitamin D is sunlight but exposure of SLE patients to UV rays has been shown to exacerbate disease pathology. Studies in various populations have shown an association between low vitamin D levels and higher SLE disease activity. Vit D is a steroid hormone and also a vitamin that regulates calcium metabolism and bone health. Not until recently, its immunomodulatory role was known. Vit D receptors are widely distributed in different tissues and notably in all immune cells. Vit D deficiency/insufficiency is widespread in the world due to life style changes: 90% is available from sunlight. SLE is a disease driven by a cytokine IFN α, and there were evidences that Vit D modulates IFN α and its signature genes. This study was aimed to observe this correlation. We enrolled 129 patients who fulfilled American College of Rheumatology criteria in the study. There were 79 treatment-naïve cases and 50 patients who were under treatment for underlying SLE. There were 100 healthy subjects from similar geographical areas includedas controls. Plasma 25-OH vitamin D_3 and interferon(IFN)-α levels were quantified by enzyme-linked immunosorbent assay (ELISA). The gene expression level of IFN-αwas determined by quantitative real-time reverse transcriptase polymerase chain reaction (RT-PCR). Plasma 25-OH vitamin D_3 significantly correlated in an inverse manner with systemic lupus erythematosus disease activity index (SLEDAI) scores, plasma IFN-α and levels of IFN-α gene expression. Further, plasma levels of IFN-α positively correlated with gene expression of IFN-α. These results suggest an important role of vitamin D in regulating disease activity in SLE patients and the need to supplement vitamin D as an adjunct to standard care.
Recently IFN α receptor inhibitor monoclonal antibody has been approved for use in SLE. The cost is exorbitant and very few patients from low and middle- income countries can afford the treatment. On the other hand, Vit D is cheap, affordable and if the results are replicated in a RCT, which we are currently involved in, it will transform the treatment protocol of SLE.

5)H Mahato, R. Tripathy, **Bidyut Das,** AK Panda**.(2018) Association between Vit D Receptor Polymorphism and systemic lupus erythematosus in an Indian cohort. Int. Jour of Rheum. Dis 21(2):468-476.doi:10.1111/1756-185X.12345**

We have been working on the immunomodulatory properties of Vit D. It is a crucial molecule in the functioning of Vit D. Vitamin D exerts its functional effect through vitamin D receptor (VDR). Vitamin D binds to VDR and induces formation of heterodimer complexes with retinoid X receptor (RXR) and is translocated into the nucleus. Subsequently, expressions of vitamin D responsive genes are induced by binding of vitamin D-VDR-RXR hetero-complex to the promoter region of the gene known as vitamin D responsive element(VDRE). We observed that despite adequate plasma levels of Vit D its actions were suboptimal. Several studies have demonstrated an association between VDR polymorphisms and susceptibility to SLE in different populations, although the results are still inconclusive. In the present study, we investigated the association of VDR polymorphisms with SLE in a cohort of patients from Odisha. We addressed this issue by performing a hospital-based case-control study by

investigating 25-OH vitamin D levels, common VDR polymorphisms (FokI:rs2228570, TaqI: rs731236, BsmI: rs1544410 and ApaI: rs7975232)and the clinical profile in a cohort of SLE. Three hundred and thirty-one female SLE patients, diagnosed on the basis of revised American College of Rheumatology classification criteria were enrolled. Two hundred and eighty-two, age-matched, unrelated healthy females were taken as controls. Interestingly, a composite analysis of VDR polymorphisms and vitamin D status revealed higher prevalence of TaqI and FokI variants along with deficient vitamin Din lupus nephritis compared to those without renal involvement. This study made an effort to analyze the possible role of VDR polymorphism and vitamin D levels in SLE on a larger cohort of patients compared to previous studies. The observations indicate that vitamin D and VDR polymorphism may have a combined, contributory role in the genesis of SLE and in the predisposition to lupus nephritis.

6)Mahato H, Tripathy R, Meher BR, Prusty BK, Sharma M, Deogharia D, Shah AK, Panda AK and **Das BK (2019**). **TNFá promoter polymorphisms (G-238 A and G-308A) are associated with susceptibility to Systemic Lupus Erythematosus (SLE) and P.falciparum malaria: a study in malaria endemic area. Nature Scientific Reports.9:11752**

Odisha is endemic for P falciparum infection and Bancroftian filariasis. Based on our observations we believe that nematodes protects against autoimmune diseases while subjects in malaria endemic areas are susceptible to SLE. High prevalence of SLE is seen in African Americans, Hispanics and Asians, people who resided or are residing in malaria endemic areas. Malaria infection is believed to be an important selection pressure during human evolution and subjects with possible survival advantage genotypes against lethal malaria are more prevalent in malaria endemic areas.. This was true across the continents where malaria was endemic. *Plasmodium falciparum* infection is a life-threatening disease with diverse clinical manifestations. TNF-α is an important molecule that works like a double-edged sword in malaria infection. TNF-α can protect an individual against severe infection but when production is unregulated it could be damaging to the host. In SLE, although IFNα remains the most important cytokine , TNF α has been found to play a significant role. There are several reports of elevated TNF-α in SLE patients.. In a study involving SLE patients of African American, European American and Hispanic American origin, high levels of plasma TNF-α was observed and a positive correlation with IFN-α was demonstrated. Defective clearance of apoptotic bodies has been suggested to be an important factor in the pathogenesis in SLE.. TNF-α has been shown to induce apoptosis.. Elevated TNF-α in SLE patients could be one of the reasons for increased apoptosis, elevated production of nuclear debris followed by defective clearance of dead or dying cells.
Importance of TNF-α in *P. falciparum* malaria and systemic lupus erythematosus (SLE) have been demonstrated. However, association of functional promoter variants in malaria and SLE in an endemic population was lacking.. A total of 204 female SLE patients and 224 age and sex matched healthy controls were enrolled in the study. Three hundred fourteen *P. falciparum* infected patients with different clinical phenotypes were included. TNF-α polymorphisms (G-238A & G-308A) were genotyped by PCR-RFLP. Plasma levels of TNF-α was quantified. TNF-α (G-238A & G-308A) variants were associated with higher plasma TNF-α levels in SLE patients residing in malaria **endemic** areas and also in patients with severe falciparum malaria, and it could be a contributing factor in the development of SLE and susceptibility to severe *P. falciparum* infection.

7) Aditya K Panda, Balachandran Ravindran, **Bidyut K Das.(2016) CR1 exon variants are associated with lowered CR1 expression and increased susceptibility to SLE in a**

Plasmodium falciparum endemic population. Lupus Science & Medicine 2016;3:e000145. doi:10.1136/lupus-2016-000145

We continued to explore the hypothesis that patients in malaria endemic areas develop protective genotypes that are deleterious and predispose subjects to SLE under the influence of environmental trigger.

Complement receptor 1 (CR1) plays an important role in immune complex clearance by opsonisation and possibly protects subjects from development of autoantibodies. Lower CR1 expression has been associated with susceptibility to systemic lupus erythematosus (SLE). In contrast, subjects displaying lower CR1 expression are protected against severe manifestations of falciparum malaria. CR1 facilitates binding, transport and endocytosis of IC bound to complements. Lower levels of CR1 have been associated with SLE and lupus nephritis (LN) Furthermore, functional CR1 polymorphisms which are believed to alter its expression on cells have been associated with increased susceptibility to SLE in different population. CR1 seems to play an important role in pathogenesis of P. falciparum malaria by facilitating rosetting, a phenomenon by which plasmodium infected red blood cells (RBCs) bind to uninfected RBCs. Rosetting is mediated by the parasite ligand Plasmodium falciparum erythrocyte membrane protein 1 on the surface of infected RBCs which binds to CR1 molecules. Subjects who express higher CR1 are believed to develop severe pathology such as cerebral malaria due to enhanced rosette formation leading to blockage of blood flow in brain capillaries. Evolution would protect against severe malarial pathology by mutation of the pathogenic gene of CR1 which makes them susceptible to SLE.

CR1 polymorphisms (intron 27 (A>T), exon22 (A>G) and exon 33 (G>C)) were typed by PCR and restriction length polymorphism in 297 cases of female patients with SLE and 300 age-matched and sex matched healthy controls from malaria endemic areas in Odisha, India. CR1 expression on monocytes was quantified by flow cytometry. The results of the study demonstrated that common CR1 exon variants were associated with diminished CR1 expression on monocytes and increased susceptibility to development of SLE and lupus nephritis in a malaria endemic area

8)) **BK Das**, AK Panda, **(2015). MBL-2 polymorphism (codon 54 and Y-221X) and low MBL levels are associated with susceptibility to multi organ dysfunction in P. falciparum malaria in Odisha, India. Frontiers in Microbiology, 6, 778. 20**

This study was a further exploration of genes and molecules that are linked or associated with both malaria and SLE.Mannose binding lectin,a plasma protein protects host from virus,bacteria,and parasites .Deficiency in MBL levels has been associated with susceptibility to various infectious diseases including *P.falciparum* malaria.Common MBL polymorphisms in promoter and coding regions are associated with decrease in plasma MBL levels or production of deformed MBL, respectively. Malaria infection elicits both innate and adaptive immune response of the host. The innate immune system is composed of diverse pattern-recognizing receptors or soluble pathogen-recognizing molecules (PRMs) which recognize specific molecular motifs on the surfaces of virus, bacteria and parasites. Mannose binding lectin is a liver derived soluble PRMs which play an important role in the innate immune response. MBL binds to sugars on the surface of pathogenic micro-organisms and triggers the complement activation system. MBL has been shown to bind to parasite infected erythrocytes

and children deficient in MBL are prone to severe malaria indicating an important role for MBL in protection against P.falciparum infection. Interestingly, MBL is an important molecule of the innate immune system that helps in elimination of debris and immune complexes and its deficiency could trigger SLE in susceptible individuals.

In this study ,we hypothesized that *MBL2* variants and plasma MBL levels could be associated with different clinical phenotypes of severe *P.falciparum* malaria.

A hospital based study was conducted in Odisha, which is endemic to *P.falciparum* malaria.Common *MBL-2* polymorphisms(codon54,H-550L,and Y-221X)were typed in 336 cases of severe malaria(SM) [94cerebral malaria(CM),120 multi-organdysfunction(MOD),122 non-cerebral severe malaria(NCSM)] and131 un-complicated malaria patients(UM).Plasma MBL levels were quantified by ELISA.

Severe malaria patients displayed lower plasma levels of MBL compared to uncomplicated falciparum malaria. Plasma MBL levels were very low in MOD patients compared to other categories. Lower plasma MBL levels are associated with increased susceptibility to multiorgan dysfunctions in *P.falciparum* malaria.

9)AK Panda, JRParida, R Tripathy, SS Pattanaik, B Ravindran, **BK Das, (2012**). **Mannose binding lectin: a biomarker of systemic lupus erythematosus disease activity. Arthritis Res Ther. 14, R218**

SLE is characterised by increased immune complex formation which gets deposited in tissues resulting in complement activation and damage. Other than the classical and alternative complement activation, MBL plays an important role in removal of excessive debris as a result of increased apoptosis in SLE. Besides its physiological role, an excessive production or diminished levels can be utilised as a biomarker to assess disease activity. Currently anti ds DNA and low C3 and C4 are taken as markers of disease activity which are not always consistent. Elevated levels of MBL have been shown in systemic lupus erythematosus (SLE) patients. In the current study, we investigated MBL as a potential biomarker for disease activity in SLE.

In a case control study SLE patients (93 females) and 67 age, sex, ethnicity matched healthy controls were enrolled. Plasma MBL levels were quantified by enzyme linked immunosorbent assay (ELISA). Clinical, serological and other markers of disease activity (C3, C4 and anti-dsDNA) were measured by standard laboratory protocol. Plasma MBL levels were significantly high in SLE patients compared to healthy controls. MBL levels were variable in different clinical categories of SLE. Lower levels were associated with musculoskeletal and cutaneous manifestations while higher and intermediate MBL levels were significantly associated with nephritis. Plasma MBL levels correlated with systemic lupus erythematosus disease activity index (SLEDAI) , anti-dsDNA, proteinuria and negatively correlated with C3. Plasma MBL is a promising marker in the assessment of SLE disease activity.

This will be an additional biomarker to the conventional ones which have been validated

10)Panda AK, Tripathy R, Das **Bidyut K (2020).** CD14 (C-159 T) polymorphism is associated with increased susceptibility to SLE and plasma levels of soluble CD14 is a novel biomarker of disease activity: a hospital based case-control study. **Lupus,** 30(2),219-227

There is a need to explore more novel biomarkers in SLE since the conventional ones like anti ds DNA and C3, C4 are inconsistent. Biomarkers are vital to assess disease severity which helps in guiding treatment protocols. We have shown plasma MBL to be a novel biomarker both as a pathogenetic molecule as well as for assessing severity of disease. sCD14 appears to be another novel biomarker for severity assessment.

Although the exact mechanism of induction of autoimmune diseases is unknown, dysregulation of the adaptive immune system has been attributed to autoantibody production. Both innate and adaptive immune systems closely interact with one another, with adaptive immunity being controlled by innate immunity. The innate immune system can be triggered by various infectious agents which possibly play a significant role in the pathogenesis of SLE. Toll-like receptors (TLRs), present on the cell surface of innate immune cells, recognize microbial motifs called pathogen-associated molecular patterns(PAMPs) and activate the downstream signal transduction pathways. There are several lines of evidence to suggest that TLR4 by itself cannot act as a receptor for lipopolysaccharide (LPS) for the induction of downstream signaling. It requires other molecules viz. LPS binding protein (LBP), myeloid differentiation protein 2 (MD-2), and CD14 for effective signaling. Since CD14 plays a crucial role in the innate immune system and infections have been an important triggering factor for the development of SLE we investigated its role in SLE. Active SLE patients display significantly higher levels compared to patients with inactive disease. Furthermore, sCD14 levels correlate positively with disease activity scores. Since CD14 (C-159T) polymorphism up-regulates levels of sCD14, we hypothesized that the variant might be associated with an increased predisposition to SLE as well as to its severity.

Two hundred female SLE patients diagnosed on systemic lupus international collaborating clinics (SLICC)classification criteria and age, sex, matched healthy controls were enrolled in the study. Polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) method was used to genotype CD14 (C-159 T) polymorphism. Plasma levels of IFN-a, TNF-a, and sCD14 were quantified by enzyme-linked immunosorbent assay (ELISA).

Prevalence of mutant genotypes (CT and TT) and minor allele (T) of CD14 (C-159T) polymorphism was significantly higher in SLE cases compared to healthy controls. Lupus nephritis patients had a higher prevalence of homozygous mutants. SLE patients displayed significantly increased plasma sCD14, TNF-a, and IFN-a levels in comparison to healthy controls. sCD14 levels correlated positively with SLE disease activity index-2K (SLEDAI-2K) scores and 24 hours proteinuria. sCD14 is a novel biomarker that needs validation.