Study of cytokines microenvironment during autoimmune diseases in patients from Bobo-Dioulasso, Burkina Faso

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Abstract

The development of autoimmun diseases involves an intricate network of cytokines that recruit and activate TREGS/ TH17 cells. This study was aimed to compare PBMC levels of pro-inflammatory and anti-inflammatory cytokines in AID patients and non-AID controls from Bobo Dioulasso. We prospectively enrolled 17 patients who had autoimmune diseases and 17 healthy donors at University Hospital SOURO SANOU and other privates clinical, from Bobo Dioulasso, BURKINA FASO, between november 2014 and december 2015 for this cohort study. Demographic characteristics and cytokines profile: IL-2, IL-10, IL-17A, IL-21, IL-22, IL-23, TNF-α and TGF-β) were determined. We used the immunoenzymatic technology to assess the titer of cytokines.

We found that there was no significant variation of TNF-α level in normal controls and autoimmune diseases patients(P=0.09).The concentrations of cytokines anti-inflammatory such as IL-2, IL-10 and TGF-β in PBMC supernatant were significantly higher in the control group than in the group of patients with autoimmun diseases (respectively P= 0.1;0.004;0.016).The supernatant levels of IL-17A, IL-21, IL-22, IL-23 and IFN-y significantly increased in autoimmun diseases in comparison to healthy controls (respectively P=0.00001; 0.001, 0.006; 0.008 and 0.000).We also found that patients with SLE and RA exhibit increased levels of IL-22, IL-21, also, patients with RA exhibit increased levels of IL-17A. Patient with HT diseases exhibit increased levels of TGF-β. Based on the level of cytokines such as IL-17A and IFN-y, we demonstrate that the phenotype IL-17+, IFN-y+ T cell is major in AID.

We have shown that patients with autoimmune diseases from Bobo Dioulasso, Burkina Faso have pro-inflammatory cytokines produced by TH17 cells such as (IL-17A, IL-21, IL-22, IL-23 and IFN-γ) are abundantly secreted in PBMC supernatants. While anti-inflammatory cytokines in the regulatory T-cell pathway (IL-2, IL-10 and TGF-β) are poorly secreted during autoimmune processes. We also found in the study a high prevalence of the phenotype of the following TH17 (IL-17 +, IFN-γ + T cells). We propose that the therapeutic targets be directed to the phenotypes to fight AID.

Keywords: Phenotype, Cytokines, Autoimmun Diseases

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Résumé

La survenue des maladies auto-immunes implique un réseau de cytokines complexe qui recrutent et activent des cellules TREGS / TH17. Cette étude a pour but de comparer les taux de cytokines pro-inflammatoires et anti-inflammatoires dans les surnageants de PMBC chez les patients souffrant de maladies auto-immunes et chez les donneurs sains de Bobo Dioulasso.

Il s'agit d'une étude prospective de cohorte qui s'est déroulée de novembre 2014 à décembre 2015 et a concerné 17 patients souffrant de maladies auto-immunes et 17 donneurs sains. L étude s'est déroulé à l'hôpital universitaire SOURO SANOU et les autres cliniques privées de Bobo Dioulasso, BURKINA FASO. Les caractéristiques démographiques et le profil des cytokines: IL-2, IL-10, IL-17A, IL-21, IL-22, IL-23, TNF-α et TGF-β) ont été déterminés. Nous avons utilisé une technique immunoenzymatique (ELISA) pour le dosage de ces cytokines.

Nous avons trouvé qu'il n'y a pas de différence statistiquement significative entre les titres de TNF-α chez les témoins sains et les patients soufrant de maladies auto-immunes (P = 0,09). Les concentrations de cytokines telles que l'IL-2, l'IL-10 et le TGF-β dans les surnageants de PBMC sont significativement plus élevées dans le groupe témoin que dans le groupe de patients souffrant de maladies autoimmunes (respectivement P = 0,1, 0,004, 0,016). Les concentrations des surnageants en IL-17A, IL-21, IL-22, IL-23 et IFN-y ont significativement augmenté dans les situations de maladies autoimmunes en comparaison avec les témoins sains (respectivement P = 0,00001, 0,001, 0,006, 0,008 et 0,000). Egalement les patients atteints de SLE et de RA ont des concentrations élevée en IL-22, IL-21, tandis que les patients atteints de RA ont des titres élevés en IL-17A. Les patients atteints de HT ont un titre élevé en TGF-β. En se basant sur la concentration de cytokines secrétées telles que l'IL-17A et l'IFN-y, nous trouvons que le phénotype IL-17 +, IFN-y + est majoritaire dans les maladies auto-immunes à Bobo-Dioulasso.

Nous avons montré qu'au cours des maladies auto-immunes à Bobo-Dioulasso au Burkina Faso, les cytokines pro-inflammatoires produites par la voie des cellules TH17 telles que (IL-17A, IL-21, IL-22, IL-23 and IFN-y) sont secrétées en abondance dans les surnageants de PBMC. Alors que les cytokines anti-inflammatoires de la voie des cellules T régulatrices (IL-2, IL-10 et le TGF-β) sont très peu sécrétées au cours des processus auto-immuns. Aussi nous avons mis en évidence dans note étude une prévalence élevée du phénotype de TH17 suivant (IL-17+, IFN-y+). Nous proposons que des cibles thérapeutiques soient dirigées vers ces phénotypes pour lutter contre les maladies auto-immunes.

Mots-clés : Phénotype, Cytokines, Maladies auto-immunes.

Introduction

Autoimmune diseases are characterized by autoimmune reactions against one's own widespread determinants. Many cytokines are involved in activity regulation and organ involvement in various autoimmune diseases [11, 15, 22, 25]. These cytokines are synthesized particulary in autoimmune diseases such as rheumatoid arthritis, type I diabetes, systemic lupus erythematosus and multiple sclerosis, and worth stressing is the difference between cytokines as phenotype markers and cytokines as inflammation and tissue damage mediators. In most autoimmune diseases the balance between pro-inflammatory and anti-inflammatory cytokines determines the extent and spread of inflammation and can lead to conspicuous clinical effects such us autoimmune diseases [11, 19]. In SLE patients, for instance, studies showed a significant elevation of TNF-α and IL-10 in all, but especially in neurologic disease form. Understanding of the fundamental mechanisms of T cell differentiation control is the road to the strategy of cytokine phenotype modulation and prevention of tissue damage and autoimmune diseases, promoting naturally the protection from them. To understand the molecular immun in patients with autoimmun diseases, we wanted to characterize the profile of cytokines that interact with TH17 / TREGS to develop treatments biotherapy in autoimmune diseases.

Material and methods

Ethic Statement

Samples analyzed in our study derived from patients who went to hospital for consultation. Patients with autoimmune diseases enrolled in our study received effective immunonosuppressors treatment alone or in association with another drug. The study was approved by our National Ethic Committee for health research in Burkina Faso Ouagadougou. Patients participating in this study gave written informed consent.

Patients and method

We prospectively enrolled 17 patients who had autoimmune diseases and 17 healthy donors at University Hospital SOURO SANOU and other privates clinical, from Bobo Dioulasso, BURKINA FASO, between november 2014 and december 2015 for this cohort study. Demographic characteristics and cytokines profile: IL-2, IL-10, IL-17A, IL-21, IL-22, IL-23, TNF-α and TGF-β) were determined.

Laboratory assay

Nature of cells

Cytokine secretion was assayed in incubation supernatant of peripheral venous blood mononuclear cells (PBMC) from patients with autoimmune disease and healthy donors (control group). All blood samples were taken at the laboratory of the SANOU souro university hospital in Bobo Dioulasso, Burkina Faso, after approval of the study protocol by the ethics committee.

Isolation and incubation of peripheral blood mononuclear cells (PBMCs) from whole blood

In each patient, we had taken 10 mL of peripheral venous blood every morning on an empty stomach in two tubes with heparin as an anticoagulant. The whole blood was diluted to (½) in a Hank's balanced salt solution devoid of Ca2 + and Mg2 + (Gibco BRL, France) with penicillin-streptomycin 100UI/mL (Biochrom KG, Germany). Lymphocytes and monocytes cells were separated by gradient centrifugation using Ficoll-Histopaque with a density of 1.076 (Sigma) (30 min à 400g).After two successive washes, we resuspended it in RPMI 1640 medium (Gibco BRL) with 10% (v/v)of Fetal calf serum (FCS)inactivated with heat (56 °C, 30 min, Gibco BRL), and we added antibiotic (penicillin/streptomycin100UI/mL). Cells were incubated, at 2 x 10^5 cells / well of 24-well plates (Corning Inc, NY) at 37 ° C in a humidified incubator (95% air - 5% CO2). PBMC supernatant was diluted in DMSO (dimethylsulfoxide, Sigma; maximum 10% (v / v) per well), with activation by lipopolysaccharide (LPS) of *Salmonella abortus equi*, 5 .µg / mL) (Sigma).

After 24 hours of incubation, the plates were centrifuged (15 min to 200 g), and the supernatant was removed and then frozen at -80 ° C. until the Elisa assay performed in the laboratory CERBA in France.

Determination of cell viability

We addided in Malassez cell, 50µl of PBMCs and 50µL of trypan blue for the cell count. Refractive cells are those that are alive and dead cells absorb trypan blue.

$$\textbf{Cell viability (\%)} = \frac{\textit{number of living cells}}{\textit{number of living cells + number of death cells}}$$

Immunoenzymatic technology

The surpernatant from PBMC level of following cytokines: IL-2, IL-10, IL-17A, IL-21, IL-22, IL-23, TNF-α and TGF-β were determinate using Immunoenzymatic technology Elisa. Briefly, 96 well plates (Corning Inc.) were treated with 25 mL / well of a solution of the first capture monoclonal antibody (HalfMoonBay, USA) (5µg / mL) diluted in PBS 0.5% tween (Sigma). The plate is incubated overnight at 4 ° C., washed three times and the nonspecific binding sites saturated by incubation for 1 hour at room temperature with PBS 3%milk. After additional washings, we added 25µL of sample, or human recombinant cytokines used for the standard range (Pharma Biotechnologies, Germany) for h-IL-2; H-IL-10; h-IL-21; R & D Systems Europe, UK for h-IL-22; H-IL-23; 1H-IL-17A; h-TGF-β and h-TNF-α. Then, 25 µL of a biotinylated antibody solution (Antibody Solutions, Half Moon Bay, USA) (2 µg / mL) are duplicated in each well. The plates are incubated for 2 hours at room temperature. After washing 50 µL, of a solution of streptavidin -HR peroxidase (Zymed, USA) diluted 1/3000 in PBS1X are deposited in each well. After 1 hour of incubation at room temperature, the enzymatic reaction is revealed by O-phenylenediamine dichlorate (OPD SigmaFast, Sigma). Finally, we read the results of DO, using a spectrophotometer (OpsysMRDynex® Technology) set at the wave length of 450 nm. Cytokines concentration was determined by interpolation of the standard range, and the results standardized with control.

Statistical analysis

Statistical analysis was performed using SPSS version 16. Student's independent t-test was used to determine the significant difference; $P<0.05$ was considered as significant.

Results

Characteristics of the study population

The number of visits during the study period was 73950 including 17 cases of autoimmunes diseases. The referral diagnosis was right in 17/65 patients (26.2%). The incidence was 17/73950 (0.023%). In group of AID patients (n= 17), there were 16 females and 1 male with a mean age of 29.2 ± 3.2 years). The controls consisted of 17 normal healthy who were matched for age and sex with the patient group. In this group there were 2 males and 15 females with a mean age of 28.7 ± 2.3 years.

Cytokine Assay

Cytokines levels in supernatant from PBMCs, namely, IL-2, IL-10, IL-17A, IL-21, IL-22, IL-23, TNF-α, TGF-β, and IFN-y were measured in normal patients (n =17), group of AID patients (n =17) (table I). It was observed that the mean supernatant from PBMC level of TNF-α was (104.4±15.2 pg/mL) as compared to normal controls at 92.5±12.1pg/mL. There was no significant variation (table I).

The mean supernatant from PBMC level of IL-2 in control group was 78.1±9.9 pg/mL, whereas level in group of AID patients was 35.9 ± 15.8 pg/mL showing a significant variation (p =0.01).

The mean supernatant from PBMC level of TGF-β in the control group was 27.51±7.7 pg/mL, whereas level in group of AID patients was 18.02 ±5.6 pg/mL showing a significant variation (p =0.004).

The mean supernatant from PBMC level of IL-22 in the group of AID patients was 79.5±22.3 pg/mL when compared with the normal controls at 45±17 pg/mL; the variation was significant ($p < 0.006$). The mean PBMC supernatant level of IL-17A was high in group of AID patients at 239.8±87.5 pg/mL, as compared to the normal controls at 102.4±42.3pg/mL. There was also a significant increase in the group of AID patients as compared to the controls subjects ($P= 0.024$). The mean supernatant from PBMC level of IL-21 was also in control subjects 85.9± 15.6 pg/mL and 198.6±82.7 pg/mL in the group of AID patients. However, there was a significant difference ($P = 0.00001$).The mean supernatant from PBMC level of IFN-y in control subjects was 225±31.5pg/mL, but it was higher in group of AID patients at 294.2±40.1pg/mL. And this difference was significant ($P = 0.000$).

The table I show the PBMC supernatant level of TNF-α, IL-2, IFN-y, IL-10, IL-17A, IL-21, IL-22, IL-23, TGF-β and IFN-y in the Controls and group of AID Patients

Table I: PBMC supernatant level of TNF-α, IL-2, IFN-y, IL-10, IL-17A, IL-21, IL-22, IL-23, TGF-β and IFN-y in the Controls and group of AID Patients (Mean ± SD)

Cultures (Pg/mL)	Control (n = 17)	Group of AID patients (n =17) (Pg/mL)	P value (control vs. patient)
TNF-α (mean±SD)	92.5±12.1	104.4±15.2	P<0.09 (NS)
IL-2 (mean±SD)	78.1±9.9	35.9±15.8	P= 0.01 (S)
IFN-y (mean±SD)	225±31.5	294.2±40.1	P<0.000 (S)
TGF-β (mean±SD)	27.51±7.7	18.02±4.6	P<0.004 (S)
IL-22 (mean±SD)	45±17.7	79.5±2.3	P<0.006 (S)
IL-23 (mean±SD)	84.1±20.4	110±21.1	P<0.008(S)
IL-17A (mean±SD)	102.4±21.8	239.8±67.5	P<0.00001 (S)
IL-21 (mean±SD)	85.9±15.6	198.6 ±82.7	P<0.001 (S)
IL-10 (mean±SD)	266.2±25.3	212.6±22.1	P<0.016 (S)
Ratio of mean IL-2/IL-17A	0.76	0.15	T REGS/TH17

Interleukin 2(IL-2) concentration in supernatant from PBMC of control group and patients with autoimmune diseases.

We examined the potential role of IL-2 in autoimmune diseases by comparing the IL-2 titers obtained in the control group and the patient group. We find that the production of IL-2 is significantly higher in the control group than in the group of patients (Figure 1). But there was seeded no significant variation between the different autoimmun diseases ($p= 0.07$).

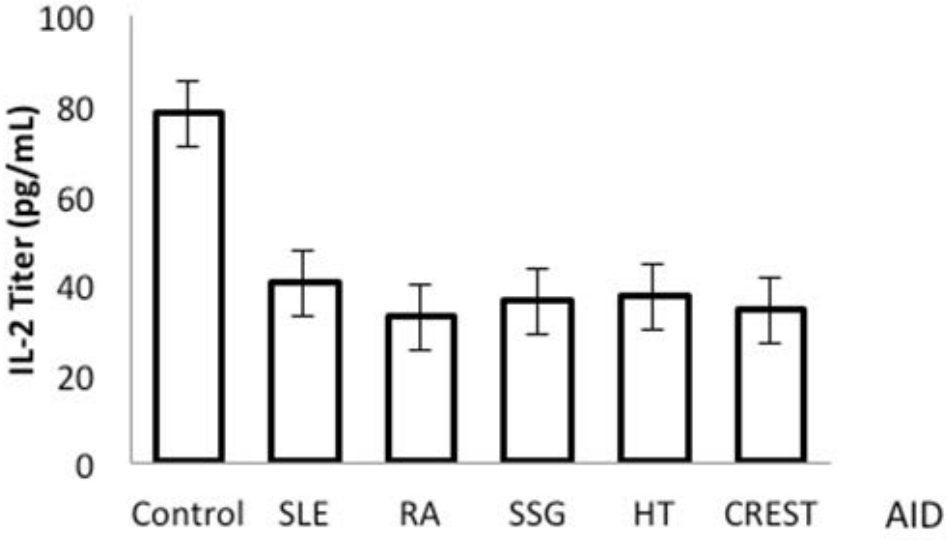

Figure 1: IL-2 concentration in the surpernatant from PBMCs at healthy donors and patients with autoimmun diseases.

Each bar reprents means± SEM (n= 17for control and n=17 for patients with autoimmune diseases.

Interleukin 10(IL-10) concentration in supernatant from PBMC of control group and patients with autoimmune diseases.

We examined the potential role of IL-10 in autoimmune diseases by comparing the IL-10 titers obtained in the control group and the patient group. We find that the production of IL-10 is significantly higher in the control group than in the group of patients (Figure 2). But there was seeded no significant variation between the different autoimmune diseases (p= 0.09).

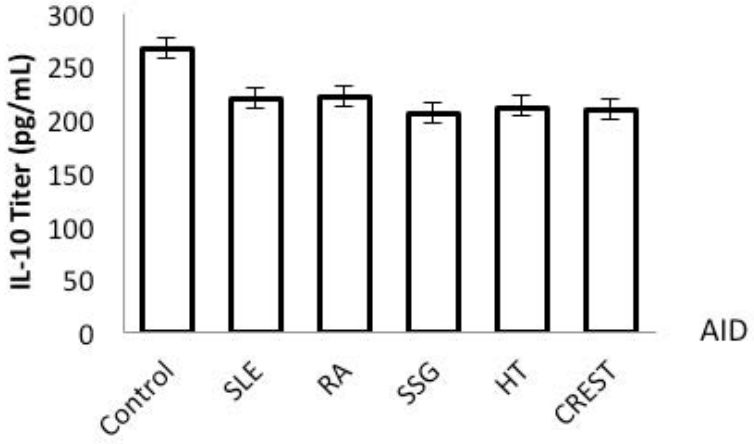

Figure 2: IL-10 concentration in surpernatant from PBMCs of healthy donors and patients with autoimmune diseases.

Each bar reprents means± SEM (n= 17for control and n=17 for patients with autoimmun diseases.

Interleukin 17A (IL-17A) concentration in supernatant from PBMC of control group and patients with autoimmune diseases.

We examined the potential role of IL-17A in autoimmune diseases by comparing the IL-17A titers obtained in the control group and the patient group. We obtained a significantly lower IL-17A titer in the control group than the patient group (Figure 3). Also, there was seeded a significant variation between patient with RA and others autoimmun diseases (p= 0.001).

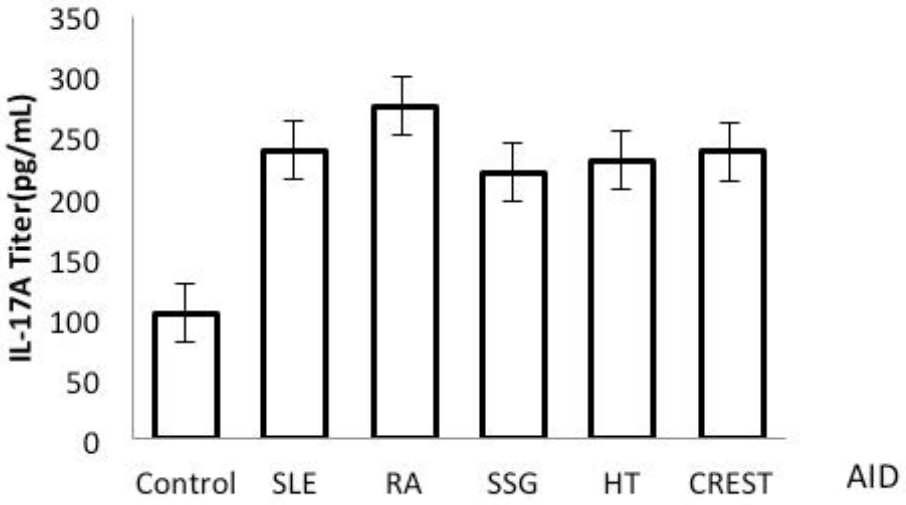

Figure3: IL-17A concentration in surpernatant of PBMCs of healthy donors and patients with autoimmune diseases.

Each bar reprents means± SEM (n= 17for control and n=17 for patients with autoimmun

TGF-β concentration in surpernatant of PBMCs of control group and patients with autoimmune diseases.

We examined the potential role of TGF-β in autoimmune diseases by comparing the different titers obtained in the control group and the group of patients. We obtained a significantly higher TGF-βtiter in the control group than the patient group (Figure 4). There was seeded a significant variation between patients with HT disease and the others autoimmun diseases (p= 0.03).

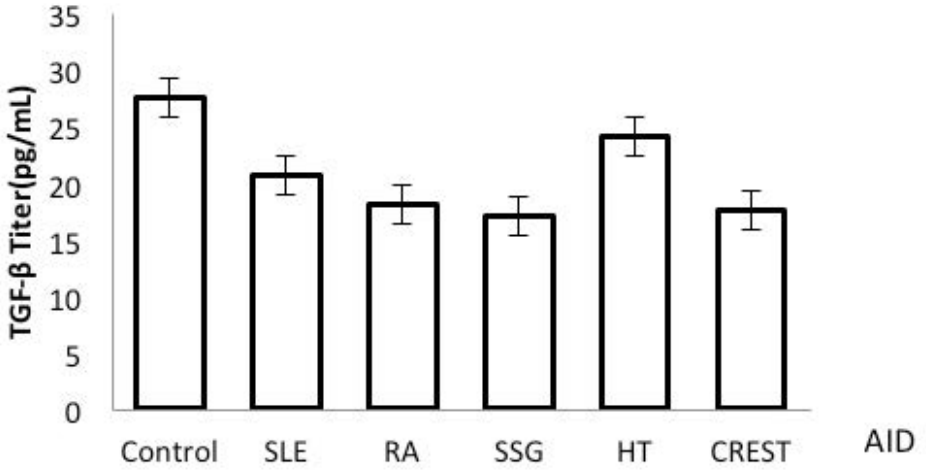

Figure 4: TGF-β concentration in surpernatant from PBMCs of healthy donors and patients with autoimmune diseases.
Each bar reprents means± SEM (n= 17for control and n=17 for patients with autoimmune diseases.

Interleukin 22(IL-22) concentration in surpernatant from PBMCs of control group and patients with autoimmune diseases.

We examined the potential role of IL-22 also in autoimmune diseases by comparing the IL-22 titers obtained in the control group and the patient group. We obtained a significantly lower IL-22 titer in the control group than in the patient group (Figure 5). Also, there was seeded a significant variation between patient with RA and others autoimmun diseases (p= 0.004) (Figure 4).

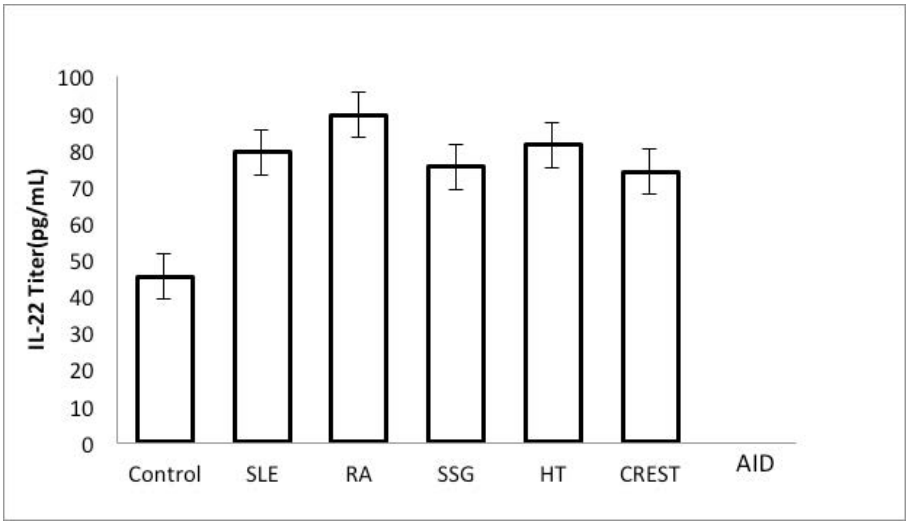

Figure 5: IL-22 concentration in surpernatant from PBMCs of healthy donors and patients with autoimmune diseases.
Each bar reprents means± SEM (n= 17for control and n=17 for patients with autoimmune diseases.

Interleukin 23(IL-23) concentration in surpernatant from PBMCs of control group and patients with autoimmune diseases.

We examined the potential role of IL-23 in autoimmune diseases by comparing the IL-23 titers obtained in the control group and the patient group. We obtained a significantly lower IL-23 titer in the control group than in the group of patients (Figure 6). But there was seeded no significant variation between the different autoimmun diseases (p= 0.075).

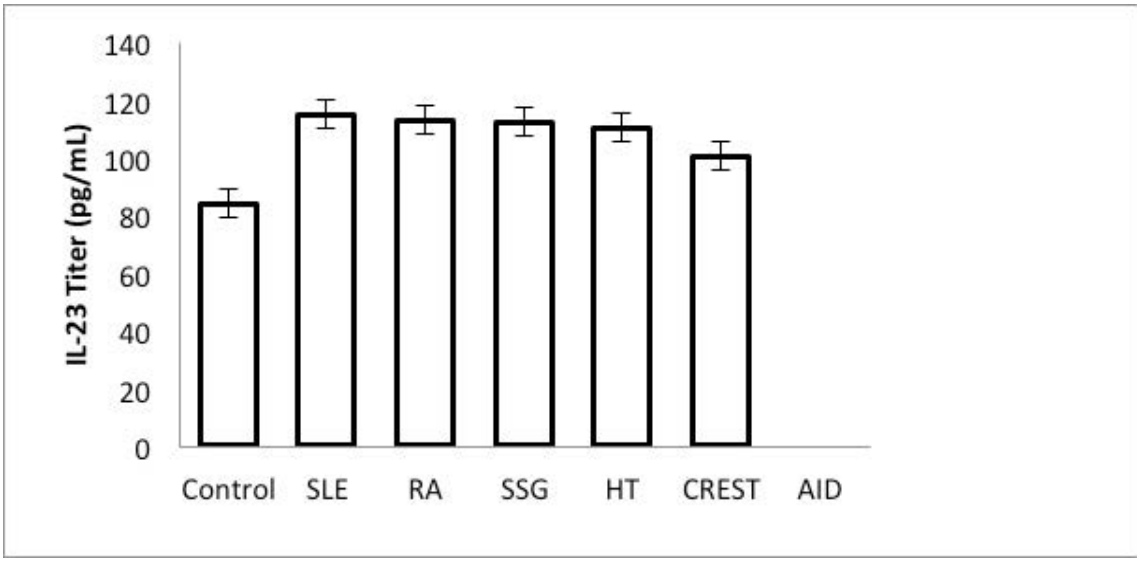

Figure 6: IL-23 concentration in surpernatant from PBMCs of healthy donors and patients with autoimmun diseases.
Each bar reprents means± SEM (n= 17for control and n=17 for patients with autoimmune diseases.

Interleukin 21 (IL-21) concentration in surpernatant from PBMCs of control group and patients with autoimmune diseases.

We examined the potential role of IL-21 in autoimmune diseases by comparing the IL-21 titers obtained in the control group and the patient group. We obtained a significantly lower IL-21titer in the control group than in the group of patients (Figure 7). Also, there was seeded a significant variation between patient with SLE; RA and others autoimmune diseases (p= 0.000) (Figure 4).

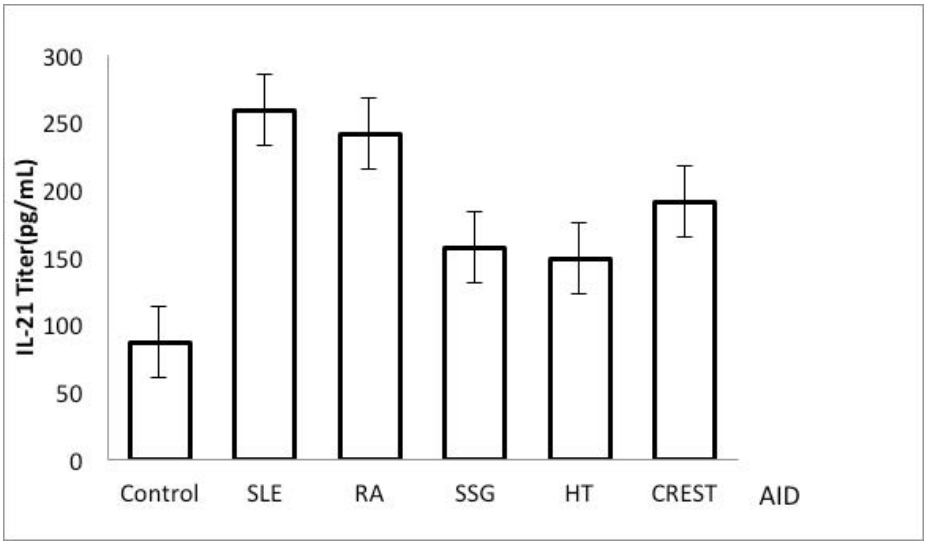

Figure 7: IL-21 concentration in surpernatant from PBMCs of healthy donors and patients with autoimmune diseases.
Each bar reprents means± SEM (n= 17for control and n=17 for patients with autoimmune diseases.

Interferon gamma (IFN-y) concentration in PBMC surpernatant of control group and patients with autoimmun diseases.

We examined the potential role of IFN-γ in autoimmune diseases by comparing the different titers obtained in the control group and the group of patients. We obtained a significantly lower IFN-γ titer in the control group than in the group of patients (Figure 8). There was seeded a significant variation between patients with SLE, RA diseases and the others autoimmun diseases (p= 0.002).

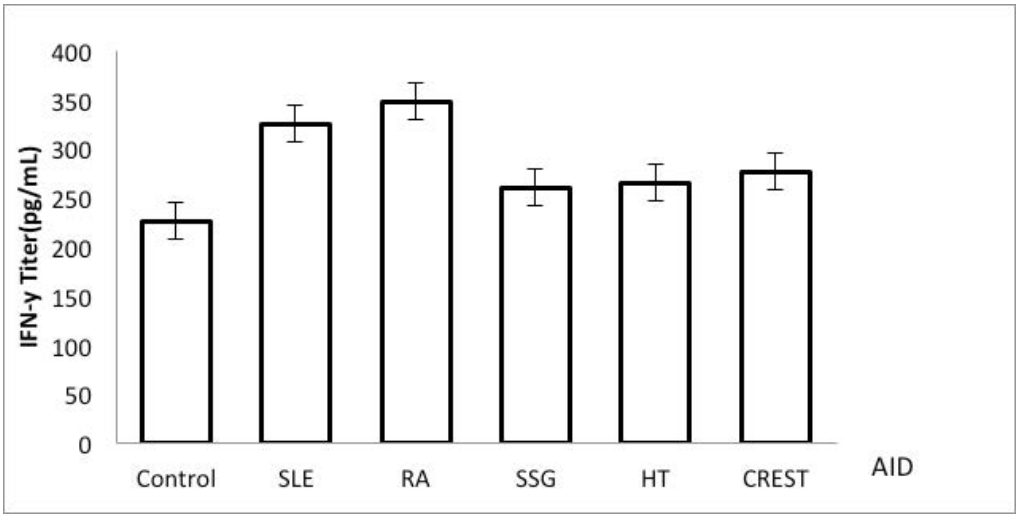

Figure 8: IFN-y concentration in the surpernatant of PBMCs of healthy donors and patients with autoimmun diseases.
Each bar reprents means± SEM (n= 17) for control and n=17 for patients with autoimmune diseases.

Discussion

We found in our study that there was no significant variation of TNF-α level in normal controls and autoimmune diseases patients. Most studies agree on a high concentration of TNF-α in autoimmune diseases [3, 25].

The difference with our results is justified by the fact that BURKINA FASO is located in an endemic or epidemic area of most tropical diseases. The transmission conditions are permanent and the immune system of the populations is also constantly stimulated by the pathogens or their vectors. Indeed TNF-α is mainly secreted by the subset TH1 cells and confirms the non involvement of these subsets of lymphocytes in the development of AID [25].

In our study, result demonstrated that IL-2, IL-10 and TGF-β levels in the patients group were significantly lower than the control group. These results agree with the findings of other studies which indicated that IL-2, IL-10and TGF-β cytokine is decreased during relapses of autoimmune diseases [4, 29].A number of studies make clear inhibitory effects of IL-10 in autoimmune diseases such as MS [3, 5].According theIL-2 and TGF-β level in PBMC supernatant, studies showed that theses cytokines induced Foxp3 expression, but both cytokines were needed to sustain expression of this transcription factor [24]. Other studies have reported that IL-2 is necessary for the survival and "fitness" of natural Treg cells [1, 6, 7, 28].

Meyer et al. reported that TGF-β1 secretion by regulatory T lymphocytes inhibit the development of EAE [18].In addition, exogenous TGF-β1 can prevent the development of EAE and the severity of the disease was increased by TGF-β1 neutralization. Also appearance of symptoms in patients with autoimmune diseases has been associated with TGF-β1 levels [20].

These findings demonstrate inhibitory role of these cytokines on the immune system cells. A defect in activation of TREGS cells could explain the development of TH17 cells the particular in AID. Indeed, TREGS cells are normally activated by dendritic cells and secrete IL-10 and TGF-β which are anti-inflammatory cytokines.

In ours study, the results presented showed that supernatant levels of IL-17A, IL-21, IL-22, IL-23 and IFN-y significantly increased in autoimmune diseases in comparison to healthy controls.

We found in our study that patients with SLE and RA exhibit increased levels of IL-22, IL-21, also, patients with RA exhibit increased levels of IL-17A. Patient with HT diseases exhibit increased levels of TGF-β. Similar results haves been showed by many studies [14, 29].

In patients with SLE,MS,RA and in experimental models, IL-17 in coordination with IL-21 and BAFF promotes germinal center formation and influences B cells to produce pathogenic autoantibodies [6,10,16].The genetic association of SLE with polymorphisms of Th17-encoding molecules (eg, IL-21) or their receptors (eg, IL-21R) has been reported [23,27].In addition, genetic variants of transcription factors (eg, ETS1) that negatively regulate Th17 differentiation can predispose to SLE [12]. Blocking of the IL-21 pathway ameliorates the autoimmune symptoms in a mouse model of SLE [8].

Recently accumulated evidence has indicated that IL-22 also plays an important role in the pathogenesis of many autoimmune diseases. IL-22 is a major cytokine in several autoimmune diseases, such as psoriasis, rheumatoid arthritis (RA), hepatitis, graft versus host disease (GHVD) and allergic diseases, implicating that target IL-22 may have a therapeutic potential in those autoimmune diseases [30].

Based on the level of cytokines such as IL-17A and IFN-y, we demonstrate that the phenotype IL-17+, IFN-y+ T cell is major in our study. Studies showed that subset Th17 cells with mixed phenotypes are observed in the peripheral blood and inflamed tissues. These findings imply that Th17 cells are unstable, and, depending on the type of inflammation and cytokine environment, Th17 cells can acquire a phenotype of other T-cell subsets, such as IL-17+, IFN-y+ T cells and IL17+, IL-4+, IFN-y- T cells. Based on more recent data obtained in IL-17A fate-reporter mice, [9, 28] Th17 cells appear to be stable under acute inflammation, whereas under chronic inflammation, these cells are vulnerable to obtain a mixed phenotype.

We propose that therapeutic targets can be directed to these phenotypes to fight autoimmune diseases.

Our study showed that supernatant from PBMC level of IL-23 was significantly different between the two groups. High levels of IL-23 are associated with increasing IL-17 secretion from T cells. Vaknin-Dembinsky et al. obtained the similar results those monocyte-derived dendritic cells in autoimmun diseases patients produce greater amounts of IL-23 compared with healthy controls [2, 26].In addition, it has reported that IL-23 and IL-17 cytokine are crucial in the pathogenesis of the autoimmun disease. Indeed, mice deficient in IL-23p19 or IL-12p40 are resistant to EAE as well as collagen-induced arthritis (CIA), another AID. These data also demonstrated that Th17 cells can therefore be described as pro-inflammatory cells, involved in many autoimmune diseases [13, 21].

Conclusion

We have shown that patients with autoimmune diseases from Bobo Dioulasso, Burkina Faso have pro-inflammatory cytokines produced by TH17 cells such as (IL-17A, IL-21, IL-22, IL-23 and IFN-γ) are abundantly secreted in supernatants from PBMC. While anti-inflammatory cytokines in the regulatory T-cell pathway (IL-2, IL-10 and TGF-β) are poorly secreted during autoimmune processes. We also found in the study a high prevalence of the phenotype of the following TH17 (IL-17 +, IFN-γ + T cells). We propose that the therapeutic targets be directed to the phenotypes to fight AID.

Author Contributions

Conceived and designed the experiments: SY SG SS TY FF. Performed the experiments: SY TY SS SG FF. Analyzed the data: SY TY.FF Contributed reagents/materials/analysis tools: SY TY FF. BW Wrote the paper: SY FF TY SG.

Conflict of Interest

We declare that we have no conflict of interest.

Abbreviations:

AID: Autoimmune Disease; ELISA: Enzyme-linked ImmunoSorbent Assay; HT: Hashimoto Thyroiditis, MS: Multiple Sclerosis, RA: Rheumatoid Arthritis, SSG: Gougerot-Sjögren' syndrome, SLE: Systemic lupus erythematosus; TGF-β: Transforming Growth Factor-beta, TNF-α: Tumor Necrosis Factor.

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