ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. LXIII, N 2, 15, 2008 SECTIO D DOI: 10.2478/v10079-008-0055-6

[1]Department of Clinical Immunology, [2]Department of Endocrinology, Medical University of Lublin
[3]Department of Hematooncology, Medical University of Warsaw

PIOTR PRZYBYLSKI[1], MARIA KUROWSKA[2], MAGDALENA GLAZER[3], DOROTA PLEWIK[1], SEBASTIAN RADEJ[1], KRZYSZTOF WIKTOR[1], JACEK TABARKIEWICZ[1], JACEK ROLIŃSKI[1]

Analysis of blood dendritic cells and lymphocytes in patients with autoimmune polyglandular syndromes (APS) and isolated autoimmune endocrine diseases – a pilot study

The immune system represents a highly effective and dynamic network that can protect a host from pathogens. Immunological self tolerance, peripheral and central, is critical for the prevention of autoimmunity and maintenance of immune homeostasis (1). It is well known that lymphocytes T compartment is responsible for regulation and balance of immune response. Breakdown of immunotolerance in T cells is thought to be connected with development of many autoimmune disorders (1, 2). Autoimmune polyglandular syndromes (APS) are rare immune-polyendocrinopathies characterized by coexistence of at least two endocrine glands insufficiency as well as the failure of nonendocrine organs, based on autoimmune mediated mechanisms (3). In 1980 Neufeld and Blizzard (4) suggested a classification of APS, based on clinical grounds indicating the four main types. The onset of autoimmune endocrinopathies is multifactorial in character and the factors include genetic predisposition, external etiological factors and disorders of the regulation in the microenvironment of target organs. However, only in type I (also known as autoimmune polyendocrinopathy, candidiasis and ectodermal dystrophy, APECED) mutation of a single gene, termed the autoimmune regulator (AIRE) gene, is responsible for loss of tolerance and immune-mediated destruction (2, 3, 5). In other types pathogenesis remains still unclear.

The aim of our study was to examine lymphocytes compartment and blood dendritic cells in patients suffering from APS.

MATERIAL AND METHODS

The study population consisted of 10 patients (9 – F, 1 – M) with APS (2 – type I, 2 – type II and 6 – type III), mean age of individuals was 47.6 years (range 21 to 71), 5 patients with isolated Addison disease (AD) (3 – F, 2 – M), mean age 43 years, (range 28 to 58), and 5 patients with isolated Graves disease (GD) (4 – F, 1 – M), mean age 46 years, (range 32 to 60) – isolated autoimmune endocrinopathy diseases (IAED). All APS, AD and GD patients were diagnosed by clinical and laboratory parameters, including hormone levels and autoantibodies against targeted tissue (data not shown). All patients exhibited different autoimmune diseases, also from other organs (Table 1). At the moment of blood examination the patients were treated with hormone replacement therapy. Healthy donors group consisted of 15 healthy volunteers (13 – F, 3 – M), mean age was 41 years (range 25 to 57) – control group (HD). 10 ml peripheral blood samples were obtained by venous puncture and

collected in sterile heparinized tubes. The blood was diluted 1:1 with phosphatase buffered saline (PBS, Biochrome, Germany) without Ca^{2+} and Mg^{2+}. Peripheral Blood Mononuclear Cells (PMBC) were isolated by Gradisol L (Aqua Medica, Poland) density gradient centrifugation. Interphase cells were removed, washed twice in PBS. For immunofluorescence studies phenotype of isolated PMBCs, panel of FITC, PE or Cy-Chrome conjugated monoclonal antibodies (BD Pharmingen, USA) were performed using: mouse anti-human BDCA-1/CD19, mouse anti-human BDCA-2/CD123, mouse anti-human CD4/CD62L/CD25, mouse anti-human CD8/CD28, mouse anti-human CD83/CD1a/ HLA-DR. Non-specific staining was inhibited by adding 10µl of FcR Blocking Reagents (Miltenyi Biotec, Germany). The labelling cells were incubated according to the manufacturers' pro and washed in buffer (PBS) afterwards. Detection of various markers was performed by using three-colour flow cytometry technique, Becton Dickinson FASCalibur flow cytometer equipped with 488-argon laser. An acquisition gate was established based on FSC and SSC that included mononuclear cells population and excluded debris. A minimum of 300,000 events was acquired. After acquisition the cells were analyzed with CellQuest Software. For statistical analysis Statistica 7.1 software was used. Results are shown as median: 25–75 percentiles, min. and max. values of percentages of positive cells in the analyzed gate.

Table 1. APS patients diseases

	APS type I	APS type II	APS type III
Addison disease	+	+ + +	
Diabetes mellitus type 1		+	+
Primary hypoparathyroidism	+	+ +	+ + +
Vitiligo	+		+ + +
Alopecia areata	+		
Mucocutaneous candidiasis	+		
Graves disease			+ +
Rheumatoid arthritis		+	
Diabetes mellitus type LADA			+ + + +
Pernicious anemia (Addison-Biermer)			+ +
Gonadal failure	+		
Asthma brionchale	+		

RESULTS

In this study we focused on lymphocytes subpopulations and blood dendritic cells (DCs) in blood from APS patients. We found that $CD8^+/CD28^+$ among $CD8^+$ lymphocytes subpopulation present lower (p=0.007) level (median= 3.85; 1.81–4.66) in patients with APS when compared with healthy donors (median= 4.6;6.63–9.31) (Fig. 1). According to the analyzed data $CD4^+/CD62L^+/CD25^-$ among $CD4^+$ lymphocytes tend (p=0.2223) to be higher (median:17.79; 3.59–26.68) than in control group (median:2.54; 1.01–30.93). In the analysis of the group of patients with APS we found significantly (p=0.000475) lower level (median= 2.46; 1.05–3.49) of CD19 positive cells when compared with HD group (median= 6.61; 4.90–8.02) (Fig. 1). On the basis of BDCA-1, BDCA-2, CD123 and CD19 antigens we recognized blood DCs. There was no significant difference in percentage of those cells between APS patients and healthy volunteers control.

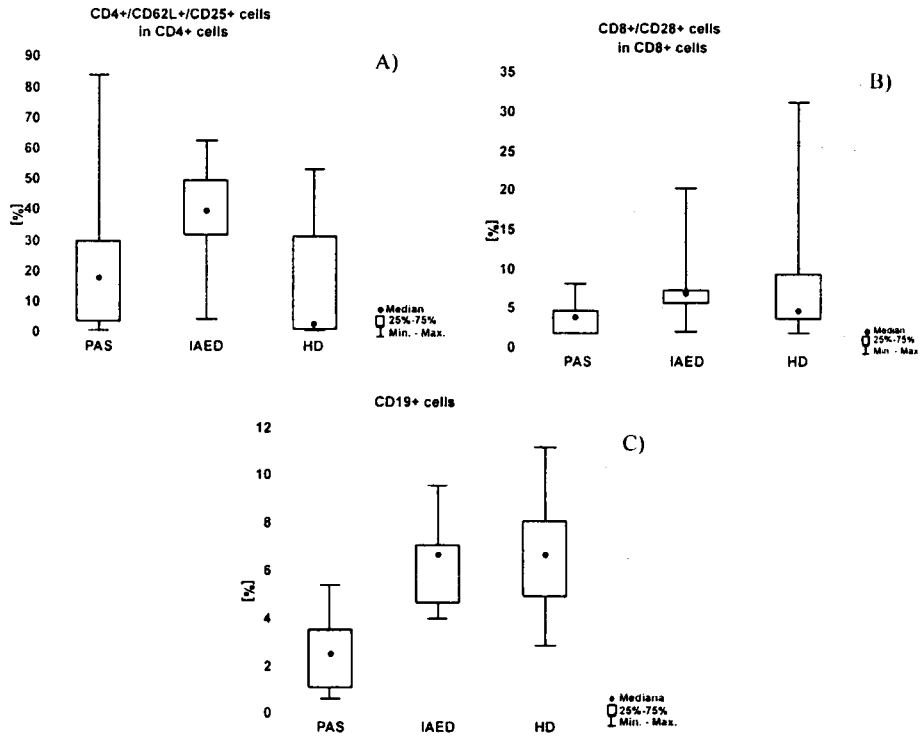

Fig. 1. Differences in lymphocytes subpopulations between patients with APS, IAED and HD group

DISCUSSION

The balance of stimulation and suppression is a sophisticated mechanism highly responsible for regulation of immune response. Disturbance of this interaction leads to development of many dysfunctions such as autoimmunity. Mechanisms of central and peripheral immunological tolerance are prevention of specific humoral and cell-mediated immune response against the constituents of the body's own tissues. Recently it has been reappreciated that the specialized subpopulation of suppressive T cells, preferentially called regulatory T cells ($CD4^+CD25^+$ T reg) play a central and prominent role in the maintenance of immunological balance (6). These T cells regulate autoagressive T and B cells and may have a profound influence on the control of autoimmune diseases (7). It was reported (8) that the main role of T reg lymphocytes is suppression of autoagressive $CD4^+$ T cells, $CD8^+$ T cells Th1 and $CD4^+$ conventional T cell toward Th2 cells. In agreement with these findings, animal studies (6) have shown that elimination, depletion or inactivation of these cells resulted in the development of autoimmunity to multiple organs, also endocrine organs, which largely resemble human APS. Persistent defective capacity of $CD4^+CD25^+$ T reg cells has been reported (1) in patients with type 2 APS but not in patients with single autoimmune endocrinopathy or healthy donors. The quantity of this subpopulation was similar in patients with type 2 and healthy donors but reduced in blood from patients with single autoimmune endocrinopathy. However, the results obtained from our study present a completely opposite situation. The highest level of $CD4^+/CD62L^+/CD25^+$ among $CD4^+$ cells was characteristic of patients from control group and the lowest – of healthy donors. The reduced level of T regulatory cells number from control patients is thought to be a manifestation of

intrinsic defect in keeping normal quantities of natural T reg in patients with single endocronopathies (1). This situation is also similar to the results obtained from animal models of autoimmunity (6). Experimental studies on Aire-deficient mice (2) and patients with type I APS have shown that in this model of APECED T reg cells develop in normal number, posses normal phenotype and function (9). Differences between T reg in human diseases and animal models are not clearly understood (2). However, the obtained data differ from those reported previously (1, 2). We suggest that the high level of regulatory lymphocytes subpopulation could be a manifestation of response to defective regulatory function of these cells. Similar situation with different T reg levels was found (2, 6) only in experimental models of autoimmune diseases depending on the animal used. Results of our findings indicate abnormal, high level of $CD8^+/CD28^+$ among $CD8^+$ cells in blood of patients from control group and the lowest in patients with APS. The previous data have shown (10) that autoreactive $CD8^+$ T cells can lead to organ-specific autoimmune diseases. The mechanism by which these cells escape tolerance induction, become activated and access the target organ remain not quite clear (10). High level of autoreactive $CD8^+$ T cells is observed in autoimmune diseases with humoral response domination like insulin-dependent diabetes mellitus (IDDM) and control group disorders, where antibodies against specific organs are produced (10). The pathological process, with production of organ-specific antibodies and progressive immune mediated destruction of endocrine tissues leads to the preclinical phase of APS (3). In the natural history of APS the absence of these antibodies does not exclude the disease, because not all patients show positive antibodies (3). According to the presented report and obtained data we could suggest that in our APS cases this type of immune response currently plays less significant role, which is reflected in lymphocytes B level. In conclusion, we described abnormal levels of T and B lymphocytes in patients suffering from APS. Disturbance in these cells populations seems to be a manifestation of destructive immune response in polyglandular endocrinopathy. However, many theories (3, 5) tried to explain specific pathology of APS. Relatively little is known about the reason why immune response in this syndrome is focused on proteins typically present in endocrine tissues and at the same time not in other organs of the same germ layer, how are these antigens selectively recognized and why multiple organs may be involved in the same individual on different occasions. Even AIRE break down and the presence of autoreactive T cells are insufficient to break tolerance (2). No autodestructive diseases occur without defect in T reg cells population (2). Nowadays only few reports (1, 2) describing defects of human regulatory T lymphocytes in APS have been published. Our findings stress the need for future investigations into better understanding of immune regulation which could be a useful target in therapy of autoimmune disorders.

REFERENCES

1. Kriegel M. A., Lohmann T., Gabler C. et al.: Defective suppressor function of human CD4+ CD25+ regulatory T cells in autoimmune polyglandular syndrome type II. J. Exp. Med., 199, 9, 1285, 2004.
2. Kakalainen E., Touvinen H., Joensuu J. et al.: A defect of regulatory T cells in patients with autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy. J. Immun., 178, 1208, 2007.
3. Dittmar M., Kahaly G. J.: Polyglandular autoimmune syndromes: long-term follow up. J. Clin. Endocrinol. Metab., 88 (7), 2983, 2003.
4. Neufeld M., Blizzard R. M.: Polyglandular autoimmune diseases. [In:] A. Pinchera, D. Doniach, G. F. Fenzi, L. Baschieri, (eds.). Symposium on Autoimmune Aspects of Endocrine Disorders. Academic Press, 357, New York 1980.

5. Betterele C., Zanchetta R.: Update on autoimmune polyendocrine syndromes (APS). Acta Bio. Medica, 74, 9, 2003.
6. Shevach E. M.: CD4+CD25+ suppressor T cells: more questions than answers. Nat. Rev. Immunol., 2, 389, 2002.
7. Jonuleit H., Schmitt E., Strassen M. et al.: Identification and functional characterization of human CD4+CD25+ T cells with regulatory properties isolated from peripheral blood. J. Exp. Med., 193, 1285, 2001.
8. Stassen M., Jonuleit H., Muller C. et al.: Differential regulatory capacity of CD25+ T regulatory cells and preactivated CD25+ T regulatory cells of development, functional activation and proliferation of Th2 cells. J. Immunol., 173, 267, 2004.
9. Anderson M. S., Venanzi E. S., Chen Z. et al.: The cellular mechanism of Aire control of T tolerance. Immunity, 23, 227, 2005.
10. Liblau R. S., Wong S., Mars L. T. et al.: Autoaggressive CD8 T cells in organ-specific autoimmunity: emerging targets for therapeutic intervention. Immunity, 17, 1, 2002.

SUMMARY

Autoimmune polyglandular syndromes are endocrinopathies based on immune-mediated destruction of endocrine tissues. In our study we examined blood dendritic cell and lymphocyte subpopulations in 10 cases of APS, 5 with isolated Addison disease and 5 with isolated Graves disease. In comparison to healthy donors significant abnormalities in the level of CD19+ and CD8+/CD28+ subpopulations were observed. The results of our study indicate abnormal levels of T and B lymphocytes in examined patients regulatory and effector cells tend to be disturbed. Conclusions. Although mechanisms of autoimmunity in APS are not clearly defined, lymphocytes seem to play the crucial role in this pathology. Understanding of pathological immune response in APS requires future investigations.

Analiza komórek dendrytycznych oraz limfocytów u pacjentów z autoimmunologicznymi zespołami wielogruczołowymi (APS) oraz izolowanymi chorobami endokrynologicznymi z autoagresji – badania pilotażowe

Autoimmunologiczne zespoły wielogruczołowe są endokrynopatiami opartymi na zniszczeniu za pośrednictwem układu immunologicznego tkanek gruczołowych. Badaniu poddano komórki dendrytyczne oraz subpopulacje limfocytów krwi obwodowej 10 pacjentów z APS, pięciu z izolowaną chorobą Addisona oraz pięciu z izolowaną chorobą Gravesa. W porównaniu z grupą kontrolną obserwowano odstępstwa w liczbie komórek subpopulacji CD19+ i CD8+/CD28+. Wyniki naszych badań wskazują na nieprawidłową liczebność limfocytów B, T oraz komórek regulatorowych w grupie badanej. Chociaż mechanizmy immunodestrukcji w APS nie są do końca wyjaśnione, limfocyty zdają się pełnić nadrzędną rolę w tej patologii. Dokładne zrozumienie autoagresji w APS wymaga dalszych badań.