

Dendritic Cells in Graves' Disease

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ABSTRAK

Sel dendritik (Dendritic Cell, DC) merupakan antigen presenting cells (APC) utama yang merangsang sel T naïve sehingga mencetuskan respons imun adaptif. Penyakit Graves' (Graves' Disease, GD) merupakan penyakit autoimun yang ditandai adanya autoantibodi terhadap Thyroid Stimulating Hormone Receptor (TSHR), yang berikatan dengan TSHR dan merangsang produksi hormon tiroid. Sel dendritik masih merupakan APC utama pada respons imun GD, meskipun sel tirosit pada GD juga mengekspresikan molekul Major Histocompatibility Class (MHC) kelas II. Penelitian mengenai DC pada GD dilakukan dengan mengisolasi DC intra tiroid maupun DC di sirkulasi perifer. Hasil penelitian DC pada GD masih kontroversial. Ditemukannya perubahan jumlah dan profil DC pada GD menunjukkan adanya perubahan aktivitas respons imun dan gangguan sel T regulator (Treg) pada GD.

Kata kunci: sel dendritik, penyakit Graves.

ABSTRACT

Dendritic cells are major antigen-presenting cells (APC) that stimulate naïve T cells, which induce adaptive immune responses. Graves' disease (GD) is an autoimmune disease characterized by the presence of autoantibodies against Thyroid Stimulating Hormone Receptor (TSHR). The autoantibodies bind with TSHR and stimulate thyroid hormone production. Dendritic cells are still the major APC in GD immune response although thyrocytes in GD can also express Major Histocompatibility Class (MHC) class II molecule. Studies about DC in GD have been conducted by isolating intra-thyroid DC or DC in peripheral circulation. Results of DC studies in GD are still controversial. Changes in number and profile of DC are found, which indicate altered immune response activity and defects of regulator T cell (Treg) in GD.

Key words: dendritic cells, Graves' disease.

INTRODUCTION

Approximately, 60-90% thyrotoxicosis is caused by GD.¹ Graves' disease is an autoimmune disorder of the thyroid gland (Autoimmune Thyroid Disease, AITD) and characterized by the production of autoantibodies. These autoantibodies then bind with TSHR and increase the synthesis of thyroid hormones. Out of the three thyroid autoantigens, i.e. the

thyroglobulin (Tg), thyroid peroxidase (TPO) and TSHR, TSHR is the major autoantigen for Graves' disease, in which approximately 80-100% patients with untreated GD have TSHR antibodies.^{1,2}

Graves' disease develops due to a complex interaction between genetic and environmental factors. Gene polymorphism, either genes associated with immune response or specific

gene for thyroid gland, can affect the incidence of GD. Environmental factors that have roles in pathogenesis of GD include physical stress, gender, radiation exposure, iodine intake and hormonal changes (post-partum period).¹

In contrast to thyroid cells in normal subjects, thyroid cell in AITD expresses MHC class II molecules ((HLA-DR, DP and DQ); therefore, it has APC characteristics and potently stimulates T cells as well as induces the adaptive immune reaction. However, in order to be able to activate the naïve T cells, in addition to the class II MHC, the costimulatory molecules are also required. Although they express the class II MHC, but the thyrocytes do not have costimulatory molecules; therefore, they can not stimulate naïve T lymphocyte cells (non-professional APC).^{1,2}

Initial immune response in GD requires two signals, i.e. the specific and unspecific signal. Specific signal comes from exogenous or endogenous Ag; while the non-specific signal is a signal of costimulatory molecule and inflammatory cytokines. Endogenous antigen may be in the form of TSHR (the major form) and dead thyrocytes, which undergo apoptosis; while exogenous Ag may include viruses or bacteria. TSH receptor experiences post-translational cleavage and results in 2 subunit structures; the subunit A is separated and will have a function as autoantigen. Autoantigen will be engulfed and processed by professional APC, i.e. macrophage and DC, into peptides. The peptides are then bound to intracellular MHC molecule into a complex molecule of MHC-peptide, which is transferred to cell surface by APC transporter. Adaptive immune responses in GD commences when DC present the complex molecule of MHC-peptide to the naïve T cell.^{3,4} In the initial phase, the T cell through the T Cell Receptor (TCR) on its surface interacts with the MHC-peptide complex on the DC (APC). After this phase, the naïve T cell needs further signal for activation process. The next step is the interaction between CD28 on the surface of T cell and costimulatory molecules of CD80 and CD86 on the surface of DC (APC). The presence of both signals (TCR binding with MHC-peptides and binding between CD28 and CD 80/CD86) will activate the naïve T cell. The activated naïve T cell will proliferate and produce cytokines,

which activate the next cascades of immune response resulting in stimulating autoantibodies, which are produced by B lymphocytes. In GD, TSHR stimulating antibodies will induce TSHR to increase the synthesis of thyroid hormones, synthesis of thyroglobulin, increase iodine intake, protein synthesis and the development of thyroid cells that may lead to hyperthyroidism.^{1, 2,5}

DENDRITIC CELLS

Dendritic cells are components of immune cells that become connectors between innate and adaptive immune responses. The number of circulating DC ranges between 0.5 - 2% of the Peripheral Blood Mononuclear Cells (PBMCs). Dendritic cells originate from multipotent hematopoietic stem cells. Based on the type of receptor on its surface, there are two types of DC. The first is mDC (myeloid DC/conventional DC), which expresses CD11c+ CD123lo or CD11clo CD123- and derived from myeloid pathway; while the second type is pDC (plasmacytoid DC), which expresses CD11c-CD123hi and derived from lymphoid pathway. Both types of DC (mDC and pDC) have some differences in several aspects, including the ability of antigen uptake, the profile of secreted cytokines, the role on immune responses and tolerance.⁶

Plasmacytoid DC are mainly found in peripheral blood and lymphoid organs, which have the ability to respond self-nucleic acid or virus through the toll-like receptor (TLR)7 and TLR-9 by producing IFN- α and other proinflammatory cytokines abundantly; therefore, they have role in autoimmune process and innate antiviral immunity. In contrast, mDCs mainly produce proinflammatory cytokines of IL-12 and TNF- α , which elicit responses to microbial stimulation. In addition to pDCs and mDCs, there are also other types of DC, i.e. the DC precursors (CD14+ monocytes) and CD1a+CD11+, the precursors of Langerhans cells.^{7,8}

After leaving the bone marrow, DC precursors enter the blood vessel and stay in several tissues waiting for Ag stimulation. In the tissues, DC precursors change into iDC (immature DC), which expresses MHC class I and class II and low-density costimulatory molecules. Immature DCs can respond to

their microenvironment and are able to have direct interaction with microorganism through pattern recognition receptors (PARP), which is similar to the TLR and subsequently capture antigen via macropinocytosis or apoptosis of cell bodies through phagocytosis. They also express inflammatory chemokine receptors such as CCR2, CCR5, CCR6, CXCR1 and CXCR2; therefore, the immature DCs can respond to inflammatory chemokines produced by immune cells at inflammatory site. When they receive appropriate chemokine stimulation, iDCs will leave the tissues and enter lymphatic circulation, then migrating to the T cell area of the lymph node, they subsequently convert to mature DC (DC) and ready to interact with T lymphocyte cells. Mature dendritic cells express MHC class I and II and high-density costimulatory molecules; thus, they have a very strong APC capability.⁸⁻¹⁰

TOLERANCE AND AUTOIMMUNITY: THE ROLE OF DENDRITIC CELLS AND T-REG CELLS

In normal condition, the body immune response can recognize and generate tolerance to self-antigens. The ability of immune response in tolerating self-antigen is elicited by a combination between the central and peripheral tolerance. The central process is characterized by diminished immature T cells in the thymus gland and the process indeed has a main role on eliminating the autoreactive T cells. Thyroid autoantigens such as TSHR, Tg and TPO are expressed by epithelial cells of thymus gland and the expression of autoantigens is controlled by Autoimmune Regulator (AIRE) gene. Autoreactive T cell stimulated by thyroid autoantigen should be destroyed or paralyzed in the thymus, but if autoreactive T cells manage to escape, then the peripheral mechanism will take over.¹

In peripheral tolerance, DCs are in steady state condition (before any infection and inflammation occurs) and they are immature. Immature DC (iDC) will circulate through the tissues and thyroid glands, capturing self-antigens or harmless foreign protein.¹¹

Several studies have shown the role of DCs on both direct and indirect mechanism of peripheral

tolerance. In direct mechanism, DCs can cause diminished autoreactive T cells by producing tryptophan metabolites or signaling through CD95. The indirect mechanism is via immature DCs, which have tolerogenic characteristic that can stimulate the Treg cells. The last mechanism is more effective for developing memory in the tolerance process and suppressing immune response (immunosuppressive) to certain antigen in long period of time.¹¹

In steady state condition, i.e. without any inflammation or infection, the iDCs will be able to recognize self antigen or weak foreign AG and subsequently develop tolerance against the (self) Ag. This stage is very essential because DC must be able to differentiate the self antigen from the strong foreign antigen, when the infection occurs someday. When there is any inflammation or infection, the environment changes due to the production of inflammatory cytokines; therefore, it stimulates iDC maturation. Mature DC is a very strong APC; therefore, the exposure of self antigen on mature DC can induce the immune response cascade, or in other words, there is failure of tolerance against self antigen.⁸ However, if the stage of tolerance process against self antigen occurs before the infection, then the immune response to this self antigen can be prevented.¹² Multiple iDC exposure on T cells may also increase the proportion of Treg cell and subsequently it may suppress the activity of T cells and B cells; therefore, the production of antibody is suppressed. In addition, Treg cells can also interrupt DC maturation so that the DC cell can not be a potent APC. This mechanism is a mechanism that protect our body against excessive immune response to self antigen.⁸

When the body fails to tolerate self antigen, then an immune response against the self antigen will develop, which is known as autoimmune reaction.⁶ Therefore, the stage of DC maturation has an important role in the tolerance and autoimmunity process. The DC maturation process, which convert DC from immature into mature form will change DC characteristics that initially capturing antigens, but was not immunogenic becomes immunogenic. Immunogenic means that it can stimulate effector T cells (CD4, CD8) and

produce cytokines that support the next serial immune response (IFN- γ).¹¹

T-reg cells also have important role in the tolerance process against self antigen and become the key of balance in immune system, including in preventing autoimmune process. There are two types of T cells, CD4 regulator/suppressor, i.e. the innate regulator T cell in the thymus gland, and several types of peripheral lymphocyte T cell (regulator T cell type 1 / Tr1, Th3 cell / cell mimicking Tr1 cell). Treg cell (TregCD24+CD25+) is produced naturally in the thymus gland. The cell can recognize foreign- and self-antigen and it is characterized by high expression of CD25. Natural Treg cell expresses CD25+ and Foxp3 in order to suppress the immune response, either by direct interaction among cells or by the assistance of IL-10 cytokines or TGF- β . Tr1 cell, which is produced in peripheral circulation from TCD24+CD25-cell, does not express Foxp3, but it can produce IL-10 cytokines and TGF- β , but it can be also suppressed by those cytokines. Th3 cell has immunosuppressive effect, which is mediated by TGF- β .^{8,13}

The role of Treg cell in the tolerance and autoimmune process is never apart from its interaction with DC. Immature and mature DCs work on different types of Treg cells. The immature DC mainly exposes self antigen and produces or stimulates the production of IL-10 and stimulates the differentiation of Tr1 cell, which produces IL-10. In contrast, mature DC during inflammation or infection will consider self antigen as foreign antigen (microorganisms). Mature DC will support the maturation and activation of Treg cell CD24+CD25+ naturally by expressing costimulatory molecules and stimulating the production of IL-12. The Treg cell CD24+CD25+ will then control the immune response which takes place due to stimulation on foreign antigen (microorganism) or self antigen. In normal condition, there is a mechanism of controlled immune response and therefore, there is no excessive activation.⁸

In autoimmune disease, including GD, there are some triggering factors, such as inflammation, hormone changes and stress that will change the environment adjacent to

DC into inflammatory environment filled with increased level of inflammatory cytokines. Therefore, in addition to antibody production, the activity of an autoimmune disease is often correlated with the concentration of inflammatory cytokines level produced by immune cells. Such environment will convert iDC into DC, which has immunogenic characteristics and therefore, can stimulate naïve T cells and induces a serial of adaptive immune response.¹⁰

Based on the abovementioned discussion, it can be seen that in normal condition, DCs undergo maturation process in line with their migration and they are able to recognize self and non-self antigen. DCs can also control immune response against self and non-self antigen by stimulating or suppressing immune response so that unnecessary immune response will not develop. The process of DC maturation has important role on the tolerance and autoimmune process.

DENDRITIC CELL IN GRAVES'DISEASE

As a major APC that has important role on the early course of GD, DC has been studied for several times since 1988. In 1988, Kabel et al conducted a study to evaluate the profile of tDC in GD and compared it with normal thyroid gland. The study involved 13 specimens of thyroid glands obtained from GD patients who had undergone surgery. It also evaluated 9 specimens of normal thyroid glands (6 specimens were obtained from laryngectomy performed for laryngeal tumor and 3 specimens were collected from autopsies).

Immunohistochemical assay was done using several monoclonal antibodies, including OKIa (class II MHC), RFD1 (active DC), L25 (DC and B cell), RIV4 (suppressor/cytotoxic T cell), Leu3ab (Th cell), FK24 (C3bi receptor, dominating myeloid cell, CD11b). OKT6 (Langerhans cells) and CLB-RAG35 (factor VIII, von willebrand factor). The study results showed that normal human thyroid gland contained fewer population of cell with single nucleus (mononuclear cell, MNC), which gave a strong positive reaction to MHC class II marker and its morphology was similar to DC, but it did not exert any reaction to RFD1 and

L25 markers. The RFD1 and L25 markers are the markers of DC characteristics in secondary lymphoid organs, which generate active reaction to immune response. It indicates that there are few of DCs in normal thyroid tissue, but they do not have active reaction to immune response and antigen processing. These DCs have a function on eliminating foreign material and damaged thyroid antigenic material to be drifted away to the lymph nodes. In thyroid glands of patients with GD, the number of DC is greater and the cells give positive reaction to RFD1 and L25 markers that show active DC to immune response including to process the Ag. Some tDCs even seemed to have interaction with lymphocytes in thyroid gland. These findings are consistent with the previous study in experimental animal (rats), which showed that there was an increased number of tDC and the process occurs prior to the emergence of anticollod antibody in the circulation.¹⁴ It is akin to explaining the serial of general immunology process, i.e. tDC capture the intrathyroidal Ag (functioning as iDD) and then take the Ag to lymph nodes to be presented on T cells (functioning as DC). The process of DC migration is then followed by expansion and maturation of T cells and B cells in the lymphatic vessels and it even infiltrates the thyroid gland.³

The profile of DC in thyroid glands of patients with Graves' disease was then studied by Molne et al using immunocytochemical assay. The study was aimed for detecting characteristics of cells in the thyroid gland expressing HLA-DR, in which the number of cells increased in GD and the cells were located adjacent to follicle epithelium. The study results demonstrated that in the thyroid gland of GD, there were cells, which exert positive reaction to RFD-1 and had DC morphology, which were seemed to have direct interaction with follicle epithelium. Considering that both HLA-DR and RFD-1 are associated with the function of APC, direct interaction between DC and thyrocyte is an essential component in the autoimmune reaction of GD.¹⁵

Although it is not easy to isolate DC from nonlymphoid gland, Croizet et al have been successfully performed DC culture on pig thyroid glands in 2000. In their study, they found 2-3%

DC population of all pig thyroid cell suspensions. The study utilized a monoclonal antibody as the DC marker, which was directed to have reaction against the human and porcine S100 β species and HLA-DR (MHC class II) and mannose receptors that were found to react with human species. Thyroid derived DC in the culture demonstrated immature DC as they express MHC class II that have a function to capture antigen. In 48 hours after the administration of TNF- α cytokines, almost 70% of immature DC converted to mature DC. The process of DC maturation in thyroid gland, in several aspects, is different from DC culture derived from precursors.¹⁶

Based on a preliminary study conducted by Kabel et al, we know that the number of intrathyroidal monocytes and dendritic cells (originated from monocyte precursors) is higher compared to healthy individuals. Dendritic cells also seemed to have a close interaction with lymphocytes; therefore, it showed the active characteristics of those cells in autoimmune process of Graves' disease. Tas et al conducted a study to have further detailed evaluation the condition of monocytes and dendritic in peripheral circulation of patients with Graves' disease, particularly in terms of polarization of monocytes and the development of clustering dendritic cells. Monocyte polarization is a process preceding the monocyte chemotaxis and in the study, it was evaluated by studying the change of monocyte morphology. While dendritic cells clustering as a response to allogeneic lymphocytes based on the number of formed clusters, i.e. one cluster usually consisted of 4-25 dendritic cells. Defects in monocyte polarization and dendritic cell clustering were reported to occur in diseases such as chronic purulent rhinosinusitis and in immunodeficiencies in cancers. Those defects are correlated with the presence of a low molecular weight factor (LMWF), a nonspecific immunoregulator which structurally resembles to p15E, a protein of retro virus. The study found a low number of polarized monocytes in patients with Graves' disease compared to those in the healthy group (mean value of 16% (9) vs 37% (8); $p < 0.01$). Moreover, the formation of dendritic cell clustering in patients with Graves' disease is also

lower than dendritic cell clustering in the healthy group (60 vs 151, $p < 0.01$). Defects in monocyte polarization and dendritic cell clustering are probably caused by a p15E-like protein, which has immunosuppressive characteristics. They assumed that this factor occurred as a contra regulator mechanism of increased intrathyroidal immune response.¹⁷

The results of study is confirmed by Quadbeck⁽¹⁸⁾ 14 years later, who observed some types of DCs in thyroid glands of patients with GD. The Quadbeck¹ study involves thyroid glands from 15 patients with GD who had undergone surgery. Monoclonal antibody markers used in the study are CD4 marker (T4, Leu-3) (CD4), markers for Th cell / DC type, monocytes, macrophage (CD40, CD50, gp50, ICAM-3), markers for DC, monocytes, granulocytes, T cells and B cells (CD54, ICAM-1), markers for cells activated by cytokines (CD80, B7.1, BB1), markers for DC, activated B cells and macrophage (CD83, HB15), markers for DC, Langerhans cells, B cells (weak) (HLA-DR, -DP and -DQ) and facultative markers by epithelial cells (RFD1). The study results show that three populations of tDC were found, i.e. 1) immature tDC (MHC-II+/CD40-/CD80-) located in perifollicular area of the thyroid gland; 2) partially mature CD80+ tDC in the connective tissue and interstitial cluster and 3) mature tDC (MHC II+/CD40+/CD80+ RFD1+) in the cluster and adjacent to / interact with activated Th cells (CD4+/ MHC-II+).

Immature tDCs were also found in vein capillary vessels. The number of immature tDC in patients with GD is greater compared to healthy subjects and patients with toxic adenoma (toxic goiter, TG), i.e. 95%; 55% and 51%, respectively. This phenomenon finding indicates that tDC in some patients with GD has experience maturation in the form of clusters (groups), which resemble to lymphoid tissue. It is confirmed further by the expression of MHC class II, CD40, CD50, CD80 dan RFD1 of the tDC. The maturity of tDC in the cluster is also confirmed by a strong interaction between tDC and CD4+/MHC class II of Th cells. Increased expression of HLA-DR in thyrocytes probably indicates the presence of collaboration between

DC, macrophage and thyrocytes to induce autoimmunity in GD.¹⁸

The correlation between DC, thyrocytes and the effect of TSH stimulation has also been studied by Croizet et al who conducted DC culture along with thyrocytes in an experimental animal study. After 8 days, the DC population in the culture, due to the presence of TSH, increases 2-3 folds; while the number of DC population without TSH has reduced 75%. Moreover, with the presence of TSH, DC expresses mannose receptor and MHC class II on the cell surface. But when there is no TSH, DC does not express mannose receptor and MHC class II after the second day of culture. A large population of DC without any thyrocytes can not respond to TSH. The study indicates the role of TSH, the main signal for growth and differentiation of thyroid cells, to control the development and functional changes of thyroid-derived DC (DC that is found together with thyroid cells). The regulator function of TSH on DC requires the presence of thyrocytes. GM-CSF and TGF β produced by thyrocytes are the intermediate cytokines between thyrocytes and DC in response to TSH. The study also showed that thyrocytes maintain the immature population in the thyroid gland.¹⁹

The DC profile in peripheral circulation and its correlation with Treg cells in GD has been studied by Mao et al. The study involved 77 patients with untreated Graves' Disease (uGD), 13 patients with GD who had achieved euthyroid level using antithyroid agents (euthyroid Graves' Disease, eGD), 15 patients with HT and euthyroid level (euthyroid hashimoto, eHT) and 74 subjects in the healthy control group. For Treg cells marker, they used CD4+CD25hiFoxp3+. The study showed that compared to the control group, the proportion of Treg cells in uGD group was lower ($1.57 \pm 0.67\%$ vs $3.36 \pm 0.96\%$; $p < 0.001$); however, it did not apply for eGD group ($2.98 \pm 0.88\%$) and eHT group ($3.09 \pm 1.02\%$). Although there was a reduced number, but the function of Treg cells in uGD was maintained. Moreover, the proportion of Treg cells was also negatively correlated to the concentration of TSHR antibody level in uGD ($r = -0.735$; $p < 0.001$). The proportion of costimulatory molecules of CD86, CD80 and CD40 increased in uGD

compared to the control group. Untreated GD (uGD) had the highest proportion of pDC significantly compared to the control group ($41.95 \pm 8.39\%$ vs $32.85 \pm 8.02\%$; $p < 0.001$), eGD group ($31.63 \pm 7.07\%$; $p < 0.001$) and HT group ($28.50 \pm 3.96\%$; $p < 0.0001$). The highest ratio of pDC/DC was found in uGD compared to other groups and the ratio had negative correlation to the Treg cell proportion ($r = -0.689$, $p < 0.001$). The phenomenon showed that DC profile converted to pDC in uGD, which might have role in reduced Treg cells. Plasmacytoid DC (pDC) is the main IFN- α producer; therefore, increased proportion of pDC would also elevate the concentration of IFN- α cytokines in uGD. The mechanism of reduced Treg cells in uGD is due to the process of apoptosis with IFN- α dependent mechanism. The study also indicated that T3 thyroid hormone will improve apoptosis of Treg cells in uGD. The Mao study may expand the horizon of knowledge on the mechanism of Treg cell defects in patients with Graves' disease.²⁰

While Mao studied about the changes of pDC and mDC proportion in autoimmune thyroid disease, including the Graves' disease, Leskela studied the distribution of pDC in autoimmune thyroid disease.

The Leskela study involved 35 patients with GD, 49 patients with HT and 34 control subjects. The study demonstrated reduced number of pDC in peripheral blood vessels (particularly in patients with severe GD); but the number of pDC in the thyroid gland increased. Plasmacytoid DC has a role in the autoimmune disease course by increasing the production of IFN- α cytokines that may affect the maturation of immunogenic DC. Leskela showed that there was low expression of immune regulator molecule in patients with Graves' disease (ILTs/CD85, PD-L1, PSGL-1 or CD69 and IDO); therefore, there was a defect on the regulating mechanism, which resulted in a defect on the activity of T regulator cells. Changes on the DC proportion and phenotype was considered as a contribution on the GD pathogenesis.²¹

The effect of thyroid hormone on DC profile in peripheral circulation has been studied by Dedecjus involving patients with thyroid carcinoma who had total thyroidectomy surgery

(three years prior to the study).²² The study subjects had their thyroxine supplementation stopped for 5 weeks in order to achieve hypothyroid condition. After the first blood sampling, thyroxine supplementation was given again at the dose of 3-4.5 $\mu\text{g/kg/day}$. The second blood sampling was performed after 2-month thyroxine supplementation. The study was conducted to observe the effect of thyroxine on the distribution of DC subtypes in peripheral blood vessels, the capability of DC as APC (HLA-DR), to evaluate the costimulatory molecules (CD40, CD80 and CD86) as well as DC maturation (CD83) in peripheral blood vessel. The study results indicated that the thyroxine supplementation during hypothyroid state (mean TSH 57.06 ± 20.03 mIU/L) would increase both DC subtypes (mDC and pDC) at post-supplementation period (mean TSH 0.04 ± 0.029 mIU/L). Moreover, the percentage of DC expressing CD86 was higher and HLA-DR expression increased in both DC types. T3 supplementation increased CD86 expression in pDC.²²

The early process of autoimmune in GD is initiated by self antigen exposure in the form of released TSH receptor component to the professional APC (DC). The APC will present the self antigen to T cells and subsequently stimulates B cells. Next, the B cells will produce antibody against TSH receptor. In untreated GD, there is a high proportion of plasmacytoid DC and therefore, it will increase the production of IFN- α , which has role in the apoptosis of regulator T cells. The reduced number of Tregulator cells in GD plays a role on the diminished control mechanism on B cell in antibody production.

The most recent study on DC profile in patients with GD was reported by Hassan I et al in 2013. The study compared the number of plasmacytoid DC between patients with GD and patients with non-autoimmune thyroid disease (struma multinodosa). It also compared the number of pDC in the lymph nodes of neck region and in the peripheral blood of patients with GD. The study results showed that there was a greater number of pDC in lymph nodes of neck region of patients with GD compared to the neck lymph nodes of patients with struma

multinodosa. Moreover, there was a larger number of pDC in the neck lymph nodes of patients with GD than pDC in peripheral blood. It suggests that there was a migration and accumulation of pDC in the lymph nodes of neck region (draining lymph nodes).²³

Several studies that have been conducted in patients with GD showed that the number of intrathyroid DC was greater in patients with GD compared to those with other thyroid diseases or with healthy individuals. Based on cytological perspective, we know that intrathyroid DC is active for immune response and has direct interaction with thyrocytes. In patients with GD, there was an increased ratio of pDC/cDC in peripheral circulation; while pDC is a main producer of IFN- α ; therefore, there was also increased production of IFN- α , which has role in the apoptosis of T regulator cells. The low number of T regulator cells has a role in diminished control mechanism against B cell to produce antibody. In patients with GD, the number of pDC in peripheral blood is reduced; while the pDC number in the thyroid gland and lymph nodes adjacent to the thyroid gland increases. The expression of immune regulator molecules (ILTs/CD85, PD-L1, PSGL-1 or CD69 and IDO) is reduced in patients with GD resulting in a defect of regulating DC and T regulator cells. Thyroid hormone supplementation in hypothyroid condition will increase the number of peripheral DC (cDC and pDC) and increase the expression of CD86 and HLA-DR. Supplementation of T3 hormone in T reg cell culture in patients with untreated GD can increase the apoptosis of T reg cells. In the thyroid gland, the regulating function of TSH on DC requires the presence of thyrocytes. The thyrocytes will maintain DC in immature condition.

Several studies in patients with GD have demonstrated the DC profile in the thyroid gland and peripheral circulation, particularly in terms of pDC and cDC proportion. Different numbers of DC in the thyroid glands, lymph nodes and peripheral circulation, different types of peripheral DC and their correlation with the survival of T regulator cells, as well as the correlation between TSH, DC and thyrocytes

have opened greater scope of knowledge on the role of DC in regulating GD autoimmune process, both directly and indirectly through the arrangement of regulator T cells. Essential role of DC in autoimmune process has enable us to think about autoimmune response modulation using DC in the therapeutical approach for autoimmune disease, including GD.³

CONCLUSION

In GD, there are changes of profile, the number and function of DC, both intrathyroid DC and peripheral DC. The changes of DC has indirectly affected immune response by suppressing T regulator cells.

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