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Role of Dendrtic Cells in IgA Nephropathy Pathogenesis

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Abstract: Objective to discuss the possible role and mechanism of DCs in IgAN attack. Method Stimulating factors such as recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF), recombinant human interleukin-4 (rhIL-4) and tumor necrosis factor-a (TNF-a) etc. were used in vitro jointly to induce and culture DCs, a flow cytometry was used to detect expression of HLA-DR, CD83 and CDla of DCs membrane surface molecules, the MTT method was used to detect capacity of DCs of the IgAN patient group to stimulate proliferation of allogeneic T cells and the difference between the levels of interleukin 6 (IL-6) and interleukin 12 (IL-12) secreted by DCs and that of the normal control group. Result Combined application of cytokines GM-CSF, IL-4 and TNF-a is able to induce proliferation and differentiation of peripheral blood mononuclear cell into a mature dendritic cell. The surface of mature dendritic cells highly expresses human leucocyte antigen HLA-DR and surface maturity markers of relative specificity of dendritic cells, CD83 and CD1a. The capacity of DCs of the IgAN patient group to stimulate allogeneic T lymphocyte proliferation is higher than that of the normal control group and the difference has statistical significance ($P<0.05$). The capacity of DCs of the patient group influenced by lipopolysaccharide (LPS) to stimulate allogeneic T lymphocyte proliferation significantly increases compared with that of DCs of the patient group not influenced by lipopolysaccharide (LPS) and the difference has statistical significance ($P<0.05$). The IL-6 secreted by the DCs of the IgAN patient group is higher than that of the normal control group and the difference between the two groups has statistical significance ($P<0.05$). The IL-12 secreted by the DCs of the IgAN patient group is lower than that of the normal control group and the difference between the two groups has statistical significance ($P<0.05$). Conclusion DCs may regulate the balance between Thl/Th2 cells by secreting cytokines so as to play an imporat role in occurence and progression of IgAN and such factors as infection etc. may strengthen the functions of DCs thus easily triggering IgAN.

Keywords: Dendritic Cell, Phenotypic Analysis, IgA Nephropathy, Pathogenesis

1. Introduction

Dendritic cells (DCs) are the known professional in vivo antigen presenting cells (APCs) with the most powerful functions, which are able to ingest and process the antigen and express MHCI, II molecules, costimulatory molecules and adhesion molecules at a high level with characteristics [1]. They can activate initial T cells effectively and directly in vitro and vivo, promote formation of T ancillary cells and cytotoxic T lymphocytes, promote B cells generating antibodies and strengthen cellular immunity and humoral immunity functions of the organism. There they are closely associated with pathophysiological processes such as Inflammatory diseases, immune diseases, transplant rejection, tumors etc. [2].

As professional antigen presenting cells, DCs exist in the epidermal layer extensively and form a defence network, which directly act on cells of different types [3]. On one hand, DCs are able to activate initial T lymphocytes thus enabling the subtype T cells to further induce reaction of B cells [4]. On the other hand, DCs can directly act on B lymphocytes thus inducing homotype transition and promoting immune globulin generation [5]. In addition, DCs play an important role in combining the intrinsic and adaptive immune response during

the process of organism immune response due to the fact that DCs play an non-substitutable role in identifying and eliminating intrinsic and extraneous antigens thus inducing the organism to generate immune tolerance and response [6]. The regulatory mechanism of DCs during the process is seldom recognized currently. The existing studies have shown that the surface of DCs has a number of receptors, which help them the environment, detect molecular signals from extraneous antigens or their own harmful cells thus initiating corresponding immune response [7]. Conversely, the organism is not able to produce immune response even counteractive immune response, such as inhibiting activation of autoreactive T lymphocytes or inducing generation of regulatory T cells [8].

Based on the above effect of DCs in the immune response and its important role in identifying autoantigens and non-autoantigens, it is inferred that DCs play an important role in autoimmune diseases. Therefore, in recently years, more and more studies have been focusing on the role of DCs in autoimmune diseases, particularly the action mechanism in nephropathy.

2. Material and Methods

2.1. Objects and Material of Study

In the research, 60 peripheral venous blood specimens were collected in total and all of them were taken from IgAN patients seeking medical advice in our hospital from January 2012 to June 2013 and healthy volunteers. Among them are 30 cases in the IgAN patient group and 30 cases in the normal control group, which are from the health volunteers.

Recombinant human GM-CSF, recombinant human IL-4 and recombinant human were purchased from Beijing CoWin Bioscience Co., Ltd.

FITC-labelled mouse anti-human HLA-DR monoclonal antibody, FITC-labelled mouse anti-human CD80 monoclonal antibody, FITC-labelled mouse anti-human CD83 monoclonal antibody, PE-labelled mouse anti-human CD86 monoclonal antibody and E-labelled mouse anti-human CDla monoclonal antibody were purchased from Beijing Bole Bioscience Development Co. Ltd.; RPMI-1640 medium was purchased from Shanghai Baoman Biotechnology Co. Ltd.; fetal bovine serum was purchased from Beijing Bole Bioscience Development Co. Ltd.; IL-6 ELISA detection kits and IL-12 ELISA detection kits were purchased from Hangzhou Biowish biotechnology Co. Ltd.; the cryogenic refrigerator was purchased from U.S. GE Co. Ltd.; the table centrifuge was purchased from Beijing Jingli Centrifuge Co. Ltd.; the carbon dioxide incubator was purchased from LEAD-TECH (Shanghai) Scientific Instrument Co., Ltd.; the inverted phase contrast microscope was purchased from Japan 0LYMPUS Company.

2.2. In Vitro Induction Culture of Dendritic Cells

6ml was taken using a anticoagulation tube and centrifuged at 3000rpm for l0min. The supernatant blood plasma was sucked and placed in a separate container and normal saline of two times in volume was added to the lower level to dilute the blood cells. 4m1 of human lymphocyte separating medium was added to a 15ml centrifuge tube. The diluted blood cells were added gently along the centrifuge tube wall and centrifuged for 20min. The nebulous substance at the middle level is mononuclear cells. A capillary dropper was inserted into the nebulous layer to suck the mononuclear cells. They were placed in another 15ml centrifuge tube; preheated PBS of more than 5 times in volume was added; centrifuged at 2000rpm and washed for 3 times; the residual lymphocyte separating medium was removed; centrifuged at 2000rpm for 10 minutes and washed for 3 times to remove the platelets. Discard the supernatant, add RPMI-1640 culture medium containing l0% fetal bovine serum and re-suspend the cells. 20μl cell suspension was placed in a blood counting chamber and the total number of cells in the four large squares was counted. The cell concentration was adjusted to $l\times10^6$/ml and inoculated in a 24-well cell culture plate with 0.5ml/well. It was placed in a incubator containing 5%CO_2 at 37°C for culture.

The extracted peripheral blood mononuclear cells were placed in a incubator containing 5%CO_2 at 37°C for 4 hour culture. The cellular morphology was observed and the supernanant was sucked and discarded. The preheated RPMI-1640 culture medium containing no fetal bovine serum was added along the culture plate wall. The non-adherent cells were washed away gently. The remaining adherent cells were precursor cells of the dendritic cells and were cultured continuously in the culture plate after addition of 0.5ml No. 1 culture medium/well.

2.3. Induced Differentiation and Proliferation of Dendritic Cells

Observe the morphologic change of cells. No. I culture medium was added with 0.5ml/well in the culture plate to achieve 1ml culture medium per well, 50ng/ml final concentration of GM-CSF and 20ng/ml final concentration of IL-4 at the 3rd day of culture. At the 5th day of culture, the cells were collected into a 15ml centrifuge tube and then collected to the 15ml centrifuge tube after the culture plate was washed with RPMI-1640 incomplete culture medium. After being centrifuged under the condition of 1500rpm×15min and collected, the cells were re-suspended with No. II culture medium and inoculated again in the 24-well cell culture plate with 0.5ml/well after the cell concentration was adjusted to 1×10^6/ml. The final concentration of GM-CSF is 50ng/ml, the final concentration of IL-4 is 20ng/ml, and the final concentration of TNF-a is 20ng/ml. The cells were cultured continuously in the incubator and collected on the 8th day for later use [9].

2.4. Identification of Dendritic Cells

Morphological identification: The cells were observed under an inverted microscope for morphologic change during culturing.

Phenotypic analysis: The concentration of the suspension cells collected was adjusted to l×10^5/ml. 100m cell suspension and 20μ1 fluorescence labelled monoclonal antibody were added to a test tube dedicated for flow cytometry, mixed gently and kept in a dark place at 8°C-25°C for 20-30 minutes. It was washed with PBS once and centrifuged at 1000rpm for 5 minutes. The supernatant was discarded. The cells were re-suspended with PBS containing 4% paraformaldehyde and kept in a dark place at 4°C for 15 minutes. Specific antigens CD83 and CDla on the surface of dendritic cells were detected with the flow cytometry.

2.5. Phenotypic Analysis on DCs of the Normal Control Group and the IgAN Patient Group

The concentration of the suspension cells collected was adjusted to l×10^5/ml. 100m cell suspension and 20μ1 fluorescence labelled monoclonal antibody were added to a test tube dedicated for flow cytometry, mixed gently and kept in a dark place at 8°C-25°C for 20-30 minutes. It was washed with PBS once and centrifuged at 1000rpm for 5 minutes. The supernatant was discarded. The cells were re-suspended with PBS containing 4% paraformaldehyde and kept in a dark place at 4°C for 15 minutes. Antigens of CD83, CDla and HLA-DR on the surface of dendritic cells were detected with the flow cytometry.

2.6. Allogeneic Mixed Lymphocyte Reaction

Dendritic cells were collected on the 8th day of culture and re-suspend with preheated RPMI-1640 complete culture medium. The cell concentration was adjusted to 5×10^6/ml and mitomycin C was added until the final concentration reaches 25μg/m1. They were washed with RPMI-1640 incomplete culture medium for 3 times under the condition of 1500rpm×5 minutes after being treated at 37°C for 45 minutes. The above cryopreserved non-adherent cells were recovered, washed, and reserved as allogeneic T lymphocytes after the concentration was adjusted to 2×10^6/ml. They were inoculated into a 96-well plate as per the DC/T proportions of l/5, 1/10, 1/20, 1/50 and 1/100 respectively. The final volume was 200μ1/well and there were three parallel wells. The 96-well plate was placed in a incubator containing 5%CO_2 at 37°C for 5 days. 5×MTT was diluted to 1×MTT with Dilution Buffer. l00μ1 l×MTT was added to each well and incubated at 37°C for 4 hours. 150μ1 DMSO was added to each well and shaken well with a plate shaker table until crystal dissolves after the supernatant was sucked. The optical density (OD value) of each well was detected with a microplate reader at a wave length of 550nm.

2.7. Reaction of Allogeneic Mixed Lymphocyte After LPS Stimulation

When the dendritic cells of the LPS stimulation group had been cultured for 7 days LPS was added to enable the final concentration to reach lμg/ml. The cells were collected after being cultured at 37°C for 24 hours and washed under the condition of 1500rpm×5 minutes for two times. The cells were re-suspended with preheated RPMI-1640 complete culture medium. The cell concentration was adjusted to 5×10^6/ml and mitomycin C was added until the final concentration reaches 25μg/m1. They were washed with RPMI-1640 incomplete culture medium for 3 times under the condition of 1500rpm×5 minutes after being treated at 37°C for 45 minutes. The above cryopreserved non-adherent cells were recovered, washed, and reserved as allogeneic T lymphocytes after the concentration was adjusted to 2×10^6/ml. They were inoculated into a 96-well plate as per the DC/T proportions of l/5, 1/10, 1/20, 1/50 and 1/100 respectively. The final volume was 200μ1/well and there were three parallel wells. The 96-well plate was placed in a incubator containing 5%CO_2 at 37°C for 5 days. The 5×MTT was diluted to 1×MTT with Dilution Buffer. l00μ1 l×MTT was added to each well and incubated at 37°C for 4 hours. 150μ1 DMSO was added to each well and shaken well with a plate shaker table until crystal dissolves after the supernatant was sucked. The optical density (OD value) of each well was detected with a microplate reader at a wave length of 570nm.

2.8. Detection of IL-6 and IL-12 in Cultural Supernatant of DCs

The cultural supernatant was centrifuged and collected and cryopreserved at -80°C for later use before collecting the DCs which had been cultured for 8 days. The content of IL-6 and IL-12 in the two groups of cultural supernatant was detected in accordance with the instructions for ELISA reagent kit.

2.9. Statistical Analysis

The SPSS 13.0 software package was used for statistical treatment. Two independent specimens and the T test were used for analysis. $P<0.05$ indicates a significant difference.

3. Results

3.1. Morphological Identification of DCs

Under the light microscope, circular and flat adherent cells were observed after the peripheral blood mononuclear cells were cultured for 5h and the adherent mononuclear cells aggregate to form grape-like clusters of different sizes and adhere to the bottom of the culture plate, particularly the edge. The cell volume increased on the 3rd day, partial cells suspended in the cell culture solution on the 4th day, and the cell number increased and partial cells had many burrs on the 5th day. A number of dendritic large cells or cell mass floating in the culture solution on the 8th day of culture. They are dendritic cells originating from peripheral blood mononuclear cells. See Figure 1.

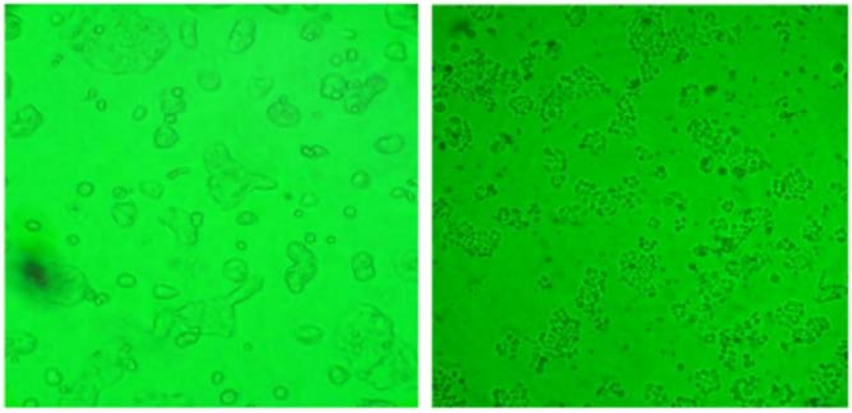


Figure 1. *Morphology of Dendritic Cells at Different Phases of Culture.*

3.2. Specific Marker on the Surface of DCs

DCs are identified mainly with CDla and CD83 molecules. CD83 marks maturity of DCs while the quantity of CDla+ cells reflects the number of DCs. With the flow cytometer, it was discovered that the research system has successfully induced CDla-cells exhibiting typical dendritic appearance and highly expressing CD83 molecules after maturity, which indicates that combined application of cell factors, GM-CSF, IL-4 and TNF-a are able to successfully induce the peripheral blood mononuclear cells to proliferate and differentiate into mature dendritic cells The surface of mature dendritic cells highly expresses human leucocyte antigen HLA-DR and surface maturity markers of relative specificity of dendritic cells, CD83 and CD1a. See Figure 2.

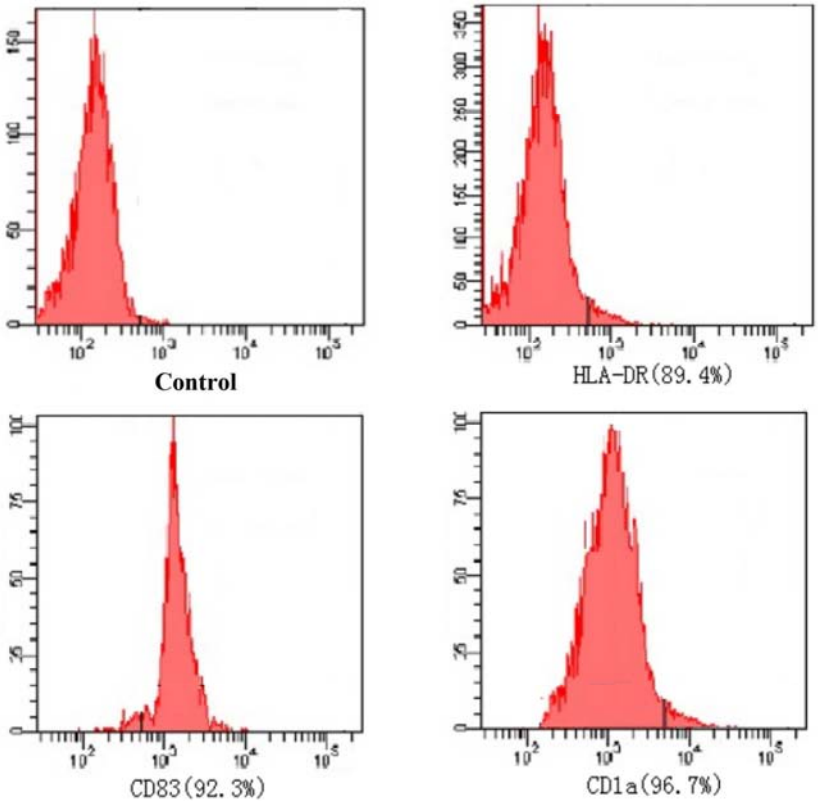


Figure 2. *Result of Detecting the Labelled Molecules on the Surface of DCs with the Flow Cytometer.*

3.3. Phenotypic Analysis on DCs of the Normal Control Group and the IgAN Patient Group

In the research, the two groups of DCs induced and cultured in vitro highly express surface molecules such as CD83, HLA-DR, CDla etc. after 6 cases of DCs from the normal control group and the IgAN patient group respectively were detected with the flow cytometer. The expression levels of the above molecules on the surface of DCs of the IgAN patient group are slightly higher than that of the control group but no

significant difference (P>0.05) has been observed between the two groups based on a statistical analysis. See Table 1.

Table 1. *Comparison of Expression Levels of Surface Molecules of DCs between the Normal Control Group and IgAN Patient Group (n=6).*

Group	CD83 (%)	CDla (%)	HLA-DR (%)
Normal Control Group	80.15±8.41	82.33±8.65	72.04±7.69
IgAN Patient Group	90.68±8.77	93.15±9.06	84.92±7.84
P Value	0.237	0.202	0.177

Legend: The normal control group and IgAN patient group express CD83, CDla and HLA-DR molecules. The level of the IgAN patient group is slightly higher than that of the normal control group but there is no statistical significance based on the comparison between the two groups, P>0.05.

HLA-DR is one of the surface MHC molecules of DCs and forms a compound with antigen peptide, which is expressed on the surface of DCs and able to present the antigen to the TCR receptors on the surface of T lymphocytes contributing to antigen presentation function of DCs. CDla and CD83 molecules are surface markers of relative surface specificity of DCs. The above result has shown that combined application of cell factors of GM-CSF, IL-4 and TNF-a in inducing and culturing DCs is able to obtain mature DCs of high purity.

CDla is mostly expressed in human glandular cells, DCs etc. and is the best marker to identify DCs. In the research, DCs are highly expressed in CDla molecules indicating that peripheral blood mononuclear cells are able to proliferate into high-purity DCs after being induced and cultured jointly by cell factors. DCs will highly express MHC molecules, costimulatory molecules and adherent molecules after maturation. Rise of CD83 costimulatory molecules is most significant while the immature DCs nearly do not express CD83. Therefore, CD83 is considered as the best mark for DC maturity.

3.4. Allogeneic Mixed Lymphocyte Reaction

In the research, it is discovered that the capacity of DCs of the IgAN patient group to stimulate allogeneic T lymphocyte proliferation is higher than that of the normal control group and the difference has statistical significance (P<0.05). The capacity of DCs of the patient group influenced by lipopolysaccharide (LPS) to stimulate allogeneic T lymphocyte proliferation significantly increases compared with that of DCs of the patient group not influenced by lipopolysaccharide (LPS) and the difference has statistical significance (P<0.05). See Table 2 and Figure 3.

The result has shown that the capacity of DCs of the patient group to stimulate proliferation of T lymphocytes is stronger than that of the DCs in the normal control group. The function enhancement of DCs in IgAN patients may be associated with the pathogenesis of IgAN. In addition, LPS is able to effectively stimulate maturation of DCs and substantially enhance their antigen presentation capacity thus stimulating proliferation and differentiation of T lymphocytes to a large extent and causing and regulating occurrence of immune response. It seemingly may explain occurrence or exacerbation of IgAN is mostly secondary to bacterial infection clinically.

Table 2. *Result Comparison for Mixed Lymphocyte Reaction.*

Group	1:5	1:10	01:20	01:50	1:100
Normal Control Group	0.36±0.08	0.30±0.06	0.25±0.07	0.22±0.05	0.16±0.05
IgAN Patient Group	0.58±0.11	0.62±0.10	0.49±0.09	0.43±0.12	0.38±0.11
LPS Stimulation Group	0.81±0.17	0.93±0.14	0.86±0.16	0.68±0.13	0.57±0.12

Legend: The capacity of DCs from the IgAN patient group to stimulate proliferation of T lymphocytes is stronger than that of the DCs from the normal control group and the difference has statistical significance (P<0.05); The capacity of DCs from the IgAN patient group to stimulate proliferation of T lymphocytes increases significantly after LPS stimulation and the difference has statistical significance (P<0.05).

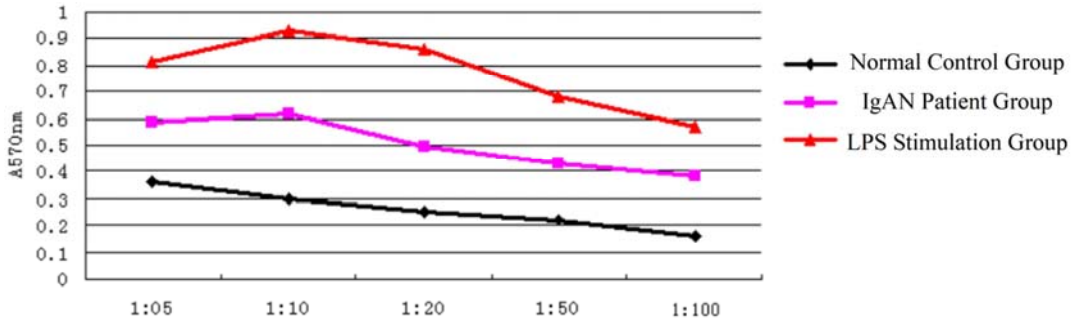


Figure 3. *Result Comparison for Mixed Lymphocyte Reaction.*

3.5. Detection of IL-6 and IL-12 in the Culture Supernatant

The experimental result has shown that the IL-6 secreted by the DCs of the IgAN patient group is higher than that of the normal control group and the difference between the two groups has statistical significance (P<0.05). IL-6 is a cell factor necessary for proliferation and antibody generation of the B cell, which promotes synthesis and secretion of the IgA antibody. The immune complex so formed are excessively deposited in glomerulus membrane domains and capillary loops leading to nephritis onset.

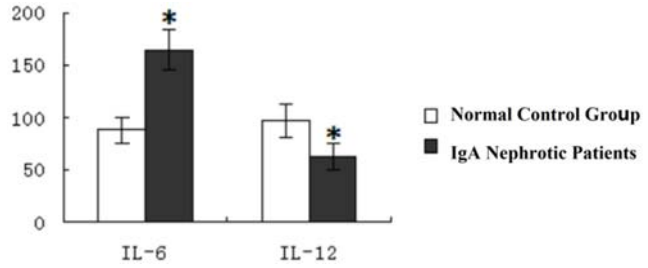
The IL-12 secreted by the DCs of the IgAN patient group is lower than that of the normal control group and the difference

between the two groups has statistical significance (P<0.05). IL-12 is currently known as the most critical cell factor with the strongest specificity inducing ThO cells to differentiate to Thl cells. DCs secrete sufficient IL-12 to induce ThO cells to differentiate to Thl cells and secrete IFN-γ to mediate cellular immune response; conversely, DCs will promote activation and proliferation of B lymphocytes of IgA molecules and thus mediate humoral immune response of the level of IL-12 secreted by DCs decreases weakening differentiation of Th0 to Thl and causing hyperfunction of Th2 cells. See Table 3 and Figure 4.

Table 3. *Comparison of IL-6 and IL-12 Secreted by DCs of the Normal Control Group and the Group of IgA Nephrotic Patients.*

Group	Number of Cases	IL-6 (pg/ml)	IL-12 (pg/ml)
Normal Control Group	30	88.37±17.51	97.35±16.82
IgA Nephrotic Patients	30	165.12±26.41	62.14±11.93
P Value		0.007	0.012

Legend: The level of IL-6 secreted by DCs of the group of IgA nephrotic patients is much higher than that of the normal control group and the difference between the two groups has statistical significance (P<0.05); the level of IL-12 secreted by the group of IgA nephrotic patients is much lower than that of the normal control group and the difference between the two groups has statistical significance (P<0.05).



Legend: Compared with the normal control group, * P<0.05.

Figure 4. *Comparison Result of IL-6 and IL-12 of the Normal Control Group and the Group of IgA Nephrotic Patients.*

4. Discussion

Based on phenotypic characteristics, DCs can be divided into two major different subtypes: medullary system DCs and plasmocyte line DCs, originating from marrow CD34+hematopoietic stem cell. The medullary system DCs are distributed two sites of the tissue, langerhans cells distributed in the epidermal layer and DCs distributed in tissue space [10]. Surface specificity of the plasmocyte line DCs expresses BDCA-2 and CDl23. The two different subtypes of DCs have different functions and their main functions primarily depend on the activation method and distribution site of DCs [11].

DCs mainly originate from marrow, peripheral blood and umbilical cord blood [12]. Bone marrow aspiration is not suitable for partial IgAN children patients bone and taking material from peripheral blood is simple, convenient and safe. Hence, the peripheral venous blood of the IgAN patients was used for induced culture and detection of dendritic cells in the experiment. DCs are almost ubiquitous in all tissues but their absolute quantity is relatively low, which only accounts for less than l% among peripheral blood mononuclear cells [13]. The low absolute quantity of DCs has long restricted relevant studies and it is possible for DCs to be applied clinically as a therapeutic schedule only after the DCs in vitro induced culture technique matures. Many scholars have been exploring the methods for DCs in vitro induced culture [14]. It is currently considered that DCs should be identified from morphological structure, surface molecule expression, functional status etc. [15].

Based on different stages of DCs, DCs can be divided into three populations: precursor DCs, immature DCs and mature DCs [16]. During in vitro differentiation, DCs can be divided into two stages: Peripheral blood mononuclear cells are differentiated into immature DCs after being induced and cultured jointly with GM-CSF and IL-4 for 3-5 days [17]. These cells have a very strong endocytosis but a weak capacity to stimulate T lymphocytes due to the fact that their surface lowly expresses costimulatory molecules (such as CD80, CD83 and CD86) and main histocompatibility complex molecules (Class MHCI and II molecules) [18]. Immature DCs will up-regulate expression of costimulatory molecules and HLA molecules and down-regulate endocytosis activity thus becoming intensive stimulating factors of T cells, i.e. mature DCs after being stimulated by stimulating factors such as TNF-a etc. for 1-2 days. The process is the maturation process of DCs [19].

GM-CSF is the most fundamental cell factor maintaining development and proliferation of DCs but only macrophages will form if peripheral blood mononuclear cells are separately added to GM-CSF [20]. IL-4 of certain concentration can be added to inhibit transformation of monocytes to macrophages and generation of granulocytes. Therefore, combined application of GM-CSF and IL-4 play an important role in inducing and culturing DCs [21]. TNF-a is able to inhibit generation of granulocytes and promote maturation of DCs to make DCs lose capacity for antigen processing and have strong activity for stimulating initial T lymphocytes [22]. Thus, it can be seen that application of GM-CSF and IL-4 can promote generation of DCs and further promote maturation and activation of DCs only after addition of TNF-a [23]. Therefore, combined application of cell factors of GM-CSF, IL-4 and TNF-a can successfully induce differentiation of peripheral blood mononuclear cells into normal mature DCs [24].

During culturing, it was discovered that many factors would influence the yield of DCs under the condition of a constant concentration of the stimulating factor. Firstly, freshness of peripheral venous blood can influence the yield of DCs. The short the in vitro time of mononuclear cells separated from a healthy human body, the higher the culture yield will be. Thus,

the number of intermediate links should be minimized and the period between separation and addition of stimulating factors should be shortened as much as possible [25]. The anticoagulant venous blood may be stored in a incubator at 37°C so as to maintain activation of mononuclear cells as much as possible if peripheral blood mononuclear cells cannot be separated timely [26]. Secondly, a number of platelets and erythrocytes are mixed when human peripheral blood mononuclear cells are separated with lymphocyte separating medium. Hence, no excessive erythrocytes should be brought in to avoid any effect on adherence of mononuclear cells when albuginea cellular layer is sucked [27]. The vast majority of platelets can be washed away after being centrifuged at a low speed (1000rpm×10 minutes) and washed with PBS for 3 times. Excessive residual platelets may also influence adherence of mononuclear cells and further reduce DCs generated if the centrifugal speed is too high or washing is improper [28]. In addition, the lymphocyte separating medium is toxic to cells. Therefore, suction of lymphocyte separating medium should be avoided as much as possible when the albuginea celluar layer is being sucked. More than 5 times volume of PBS is used to wash cells to minimize any impairment of lymphocyte separating medium on cells if lymphocyte separating medium is mixed inevitably [29]. In addition, inoculum density of mononuclear cells can also influence DC yield. Excessively high cell density can increase loss of mononuclear cells and decrease yield while excessively low cell density can inhibit aggregation and proliferation of cells [30]. In the experiment, it is discovered that the DC yield is high under the condition of $l\times10^6$/ml.

In conclusion, combined application of cell factors of GM-CSF, IL-4 and TNF-a can successfully induce proliferation and differentiation of peripheral blood mononuclear cells into mature DCs, which not only exhibit typical dendritic cell morphology but also highly express human leucocyte antigen HLA-DR and membrane surface marker molecules CD83 and CDla of DCs relative specificity after being detected with a flow cytometer. Thus, DCs obtained using the method are able to basically meet cells necessary for the follow-up test and provide bases for an in-depth study.

5. Conclusion

In the research, it was discovered that the three groups of DCs highly express such surface markers as HLA-DR, CD83 and CDla but there is no significant difference (P>0.05) between the normal group and the IgAN patient group after the surface marker of DCs of the normal control group and the IgAN patient group was detected with the flow cytometer. It indicates that combined application of cell factors of GM-CSF, IL-4 and TNF-a in inducing and culturing DCs is able to obtain mature DCs of high purity. DCs will highly express MHC molecules, costimulatory molecules and adherent molecules after maturation. Rise of CD83 costimulatory molecules is most significant while the immature DCs nearly do not express CD83. Therefore, CD83 is considered as the best mark for DCs maturity. However, in the research, the expression level of surface CD83 of the IgAN patient group is slightly higher than that of the normal control group but there is no any statistical difference (P>0.05). It indicates that CD83, as a mark of DCs maturity, may maintain and strengthen inflammatory response caused by antigens and be insufficient to regulate differentiation of the Th2 subtype.

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