Immune regulation in IgA nephropathy

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Chapter 4

Dendritic cells of IgA nephropathy patients have an impaired capacity to induce IgA production in naïve B cells

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Summary

IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide, characterized by mesangial IgA_1 deposits. We have previously demonstrated that IgAN patients have a hampered IgA immune respons after mucosal challenge with a neoantigen. Dendritic cells (DCs) are critically involved in the initiation of humoral immune responses, not only via activation of T helper cells, but also via direct effect on naïve B cells. The aim of this study was to investigate the capacity of DCs from IgAN patients to regulate IgA production.

DCs were generated by culturing monocytes for 7 days in the presence of IL-4 and GM-CSF. DCs from either IgAN patients (n=12) or controls (n=12) were cultured for 14 days with naïve B cells in the presence of CD40L-transfected mouse fibroblasts (L-CD40L cells) and medium with or without IL-2 or IL-10. Supernatants were tested for the presence of immunoglobulins by specific ELISA.

In the presense of CD40L and IL-10, DCs were able to increase immunoglobulin production by naïve B cells. DCs of IgAN patients induced significantly ($p=0.026$) less IgA production than DCs of control persons (2.30 µg/ml vs. 5.24 µg/ml), whereas no differences were found in the IgG and IgM production. When DCs were replaced by supernatant of CD40L-stimulated DCs of patients and controls, IgA production was increased, but no difference was seen between the two groups.

In the present study we show that DCs of IgAN patients have an impaired capacity to induce IgA production in naïve B cells, which might explain the observed IgA hyporesponse upon mucosal challenge with a neoantigen.

Introduction

IgA nephropathy (IgAN) is the most common primary glomerulonephritis worldwide [1-3]. Overall, IgAN is the cause of renal failure in ±10% of the people being on chronic renal replacement therapy in Western Europe and the United States. Patients usually present with microscopic hematuria and proteinuria. In renal biopsies, IgAN is characterized by IgA1 and complement component 3 (C3) deposits in the mesangium of the glomeruli [4;5]. After kidney transplantation, recurrent IgA deposits are found in the allograft in a high percentage of the cases. Moreover occasional accidental transplantation of a kidney of an IgAN patient lead to disappearance of IgA deposits [6;7]. Therefore it is most likely that the major cause of IgA deposition in the renal mesangium lies in IgA itself or the interaction between IgA and the mesangium.

The pathogenesis of IgAN is not clear. Several factors have been implicated to play a role in the formation of IgA immune deposits in the mesangium. Both quantitative and qualitative differences in IgA seem to be a factor involved in the pathogenesis [8;9]. Detailed biochemical analysis of IgA_1 suggests that underglycosylated IgA_1 is enriched both in serum and in mesangial deposits [10;11]. Moreover it has been suggested that glycosylation of IgA might be affected by the mode of B cell activation and the cytokine milieu [12].

A clinical observation is that upper respiratory tract infections frequently lead to an increase of hematuria (‘synpharyngitic hematuria’) [13;14]. Therefore a dysregulation of the mucosal immune response of IgAN patients might play a role in the pathogenesis [1]. Mucosal vaccination of IgAN patients with neoantigens was shown to lead to an impaired mucosal and systemic IgA response, compared with healthy persons [15;16]. In these studies no differences in the antigen specific IgG and the IgM responses were measured. These results seemed in conflict with the original idea that IgAN patients are hyperresponders after vaccination, as suggested by the higher serum IgA titers in most IgAN patients. The observation that systemic vaccination of IgAN patients with a recall antigen appeared to give rise to

a systemic and mucosal hyperresponse, might be explained by an increased level of immunological memory [17-19]. When we performed a simultaneous mucosal and systemic vaccination with a neoantigen, only the mucosal challenge showed a hyporesponse, whereas the systemic challenge was not different between patients with IgAN and controls [15]. This suggests that the mucosal immune regulation has specific regulatory features [20;21].

Immunoglobulin production is the result of a complex interaction between B cells, T cells and antigen presenting cells like dendritic cells (DCs). Naïve B cells are IgM/IgD positive and have to undergo a program of affinity maturation, isotype switch and plasma cell differentiation before high affinity IgG or IgA antibodies can be produced [22]. This process is tightly regulated by activated T helper cells through expression of CD40L/CD154 and the production of cytokines. In the hyper-IgM syndrome a genetic alteration of CD40L results in a deficit of circulating IgG and IgA [23;24]. For IgA production, cytokines like IL-10 and TGF-β have been shown to determine the isotype specificity [25]. More recently it has been shown in an *in vitro* model that DCs were capable to further increase IgA production in the presence of IL-10 and CD40L [26;27]. Therefore, DCs seem to have a direct effect on B cells, next to its well established role in the activation of T helper cells [28;29].

In the present study we investigated the capacity of DCs from IgAN patients to induce IgA production in naïve B cells *in vitro* and compared this with DCs from healthy volunteers. We found that DCs from IgAN patients induce less IgA production, whereas no differences for IgG and IgM were found.

Materials and methods

Selection of IgAN patients

Biopsy proven IgAN patients were selected for entry in this study. Patients did not take immunosuppressive drugs within a three months period before blood samples were taken. Age and sex matched healthy controls were selected.

No infections or macroscopic hematuria were present at the time of blood sampling.

Mean age of the patients was 42 years versus 39 years of the controls (Table 1). The male: female ratio was 83% vs 17% in both groups. Renal function was preserved and stable in most of the patients. Mean creatinine clearance was 78 ml/min. Only one patient had a creatinine clearance lower than 25 ml/min. Proteinuria was mild with a mean value of 1.3 gram per litre. The study was approved by the Ethical Committee of the Leiden University Medical Centre. All individuals gave informed consent.

Table 1. Characteristics of IgAN patients and control persons

	IgAN patients	**controls**
Number	12	12
Mean age (range)	42 (22-62)	39 (30-52)
Male/female	10/2	10/2
Years since diagnosis (range)	8 (1-22)	
Serum creatinine (μmol/ml)	135 (79-347)	N.D
Creatinine clearance (ml/min)	78 (19-117)	N.D
Proteinuria (g/L)	1.3 (0-1.9)	N.D
Erythrocyturia (0-4+)	2+	N.D

N.D is not determined

Generation of dendritic cells

Fifty ml of heparinized peripheral blood was collected from each IgAN patient and healthy control person. PBMC were isolated by Ficoll-Hypaque (Sigma, St. Louis, MO) density gradient centrifugation. Cells were washed three times, to remove Ficoll and thrombocytes. Subsequently monocytes were isolated by Percoll (Pharmacia, Uppsala, Sweden) gradient centrifugation and plastic adherence for 2 hours in six wells culture plates ($2.5x10^6$ cells/well; Costar, Cambridge, MA). Adherent monocytes were cultured for 7 days in RPMI 1640 containing 10% heat-inactivated FCS and penicillin/streptomycin (all from Gibco/Life Technologies, Breda, The Netherlands), in the presence of 5 ng/ml

GM-CSF (Leucomax, Novartis Pharma B.V., Arnhem, The Netherlands) and 10 ng/ml IL-4 (Peprotech, Rocky Hill, NJ) [30].

Analysis of cell surface phenotype by flow cytometry

Cells were harvested and washed in PBS containing 1% BSA and 1% heat-inactivated normal human serum and 0.02% NaN_3. FACS analysis was performed using mAb against CD1a (Leu-6) and CD14 (Leu-M3) (both Becton Dickinson & Co., Mountain View, CA) and CD86 (IT2.2; Pharmingen, San Diego) and CD209 (mouse anti-human DC-SIGN, AZN-DI, kindly provided by Prof. Yvette van Kooyk. Free University Medical Centre, Amsterdam, The Netherlands). Staining was visualized by using PE-conjugated goat-anti-mouse Ig (Dako, Glostrup, Denmark), assessed for fluorescence using a FACScan and analyzed with WinMDI software (Becton Dickinson & Co.).

Isolation of serum IgD+ ($sIgD^+$) B lymphocytes

B cells were isolated from tonsils by the Ficoll-Rosetting method [25]. Purified $sIgD^+$ B lymphocytes were separated using a preparative magnetic cell sorter (MACS; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany) according to the experimental procedure described in detail by Miltenyi et al. [31]. IgD was expressed on >99% of the $sIgD^+$ B cell population as assessed by FACScan analysis (Becton Dickinson & Co.).

B cell activation

All cultures were performed in Iscove's modified Dulbecco's medium (IMDM) glutamax (Gibco/Life Technologies) supplemented with 10% heat-inactivated FCS and ITS (Insulin 5 µg/ml, Transferrin 5 µg/ml and Selenium 5 ng/ml final concentration; Sigma). $1x10^4$ IgD^+ B cells were cultured in the presence of $1x10^4$ irradiated (33 Gy) DC and $0.25x10^4$ irradiated (75 Gy) murine L-cell fibroblasts stably transfected with human CD40L (L-CD40L cells) [32] in a final volume of 200 µl in 96-wells round bottom tissue culture plates (Costar) [26]. In each experiment cells were cultured in the presence of medium, IL-2 (20 U/ml) (Chiron, Emeryville, CA), or IL-10 (50 ng/ml)(Schering Plough, Dardilly, France). Each culture condition was performed in 5 fold. Conditions

without DC and/or without B cells served as negative controls. Supernatant was harvested after 14 days.

Generation of supernatant from dendritic cells

$5x10^4$ DCs were cultured for 72 hrs in combination with $1x10^4$ irradiated L-CD40L cells or $1x10^4$ L-Orient cells in a total volume of 500 µl in IMDM glutamax supplemented with 10% heat-inactivated FCS and ITS. Supernatant was harvested and stored at -20ºC until further use.

B cell experiments with supernatant of dendritic cells

All cultures were performed in IMDM glutamax supplemented with 10% heat-inactivated FCS and ITS. $1x10^4$ IgD^+ B cells were cultured in the presence of 50 µl supernatant of CD40L-stimulated or unstimulated DCs and $0.25x10^4$ irradiated (75 Gy) L-CD40L cells with or without IL-10 (50 ng/ml) in a final volume of 200 µl in 96-wells round bottom tissue culture plates (Costar). Each culture condition was performed in 5 fold. Conditions without supernatant and/or without B cells served as negative controls. Culture supernatant was harvested after 14 days and tested for Ig by ELISA.

Immunoglobulin quantification

IgA, IgA_1, IgA_2, IgG and IgM levels were determined by specific sandwich enzyme-linked immuno assay (ELISA), as described [15]. All ELISA's were performed in 96-well polystyrene plates (Greiner, Alphen a/d Rijn, The Netherlands). Optical density (OD) was measured at 415 nm on a microplate reader (Bio-Kinetics Reader EL 312e, Biotek Instruments Inc., Winooski, VT, USA).

IL-10 quantification

IL-10 was measured by a commercially available ELISA kit (Sanquin, Amsterdam, The Netherlands) and was used as indicated by the manufacturer.

Statistical analysis

Statistical analysis was performed, using students t-test, P values less than 0.05 were considered significant.

Results

Immunoglobulin production is dependent on the presence of CD40L and increased by dendritic cells

To be able to investigate the capacity of DCs of IgAN patients to modulate IgA production by naïve IgD^+ B cells, we established a culture system based on the presence of L-CD40L cells, IgD^+ naïve B cells and recombinant cytokines. Independent of the cytokines used, only very low concentrations of immunoglobulins were detected in the absence of CD40L. In the presence of CD40L the immunoglobulin production of all different isotypes was strongly upregulated (Figure 1). This Ig production is dependent on the presence of IL-10 and is not supported by IL-2. Next, monocyte-derived DCs were added to CD40L-activated B cell cultures. In the absence of exogenous cytokines, DCs did not significantly stimulate Ig production. However in the presence of IL-10 and also in the presence of IL-2 [33] DC stimulated production of IgA, IgG and IgM (Figure 2). This model allows the investigation of the B cell stimulating capacity of DCs of patients with IgAN.

No phenotypic differences between dendritic cells of IgAN patients and control persons

Immature DCs were generated by culturing monocytes from patients and controls in the presence of IL-4 and GM-CSF during 7 days. Monocytes are negative for CD1a and DC-SIGN and have high expression of CD14. Immature DCs have surface expression of DC-SIGN, a specific DC marker, and have high expression of CD1a and low expression of CD14 (Figure 3A). The phenotype of immature DC of IgAN patients and controls did not differ in expression of DC-SIGN (Figure 3B). Pilot experiments showed that individual contaminating B cells in the DC population could affect the outcome of Ig

production. This effect could be prevented by irradiation of (30 Gy) the DC population. Irradiation did not affect CD40L-induced functions of DC, like IL-10 production (Figure 4A) or CD86 expression (Figure 4B).

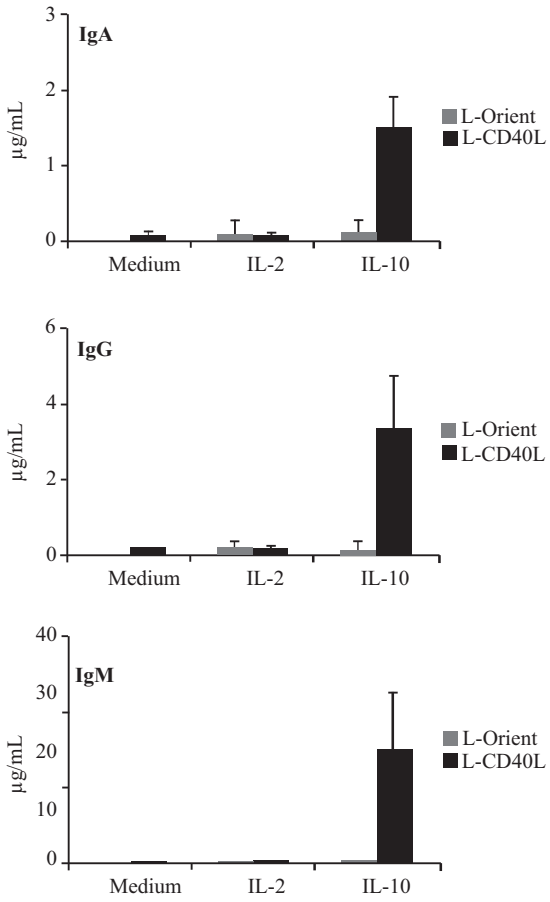

Figure 1. Immunoglobulin production is dependent on CD40L and IL-10. Highly purified IgD^+ B cells (10^4) were cultured with 75 Gy irradiated non-transfected L cells (L-orient) or CD40L transfected L cells (L-CD40L) ($0.25x10^4$) in the presence of IL-2 (20 U/ml) or IL-10 (50 ng/ml) as indicated. After 14 days supernatants were harvested and tested for IgA, IgG and IgM production using specific ELISA's. Data shown are the mean $\pm$SD of quadriple cultures and represents one out of 3 independent experiments.

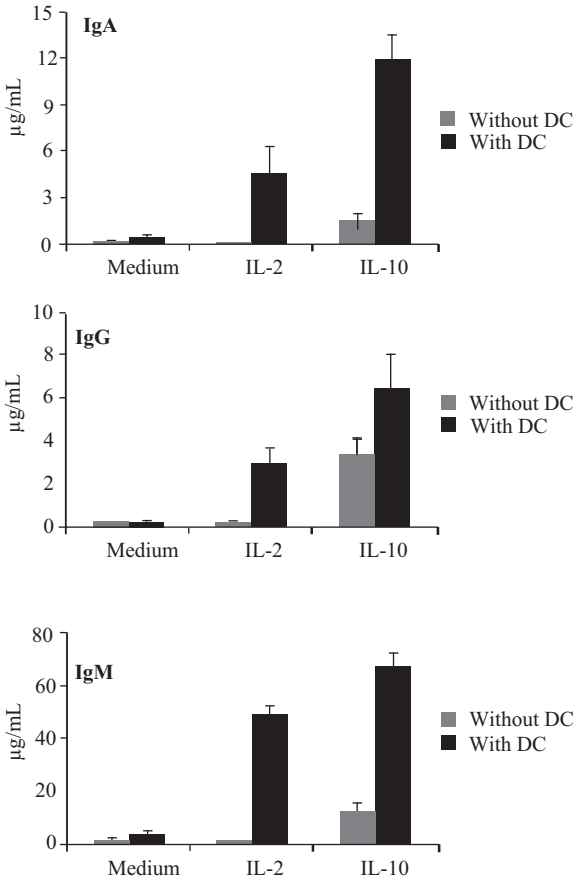

Figure 2. DCs increase Ig production by naïve B cells. Highly purified IgD+ B cells (10^4) were cultured with 75 Gy irradiated CD40L transfected L cells ($0{,}25x10^4$) in the absence of DCs (grey bars) or in the presence of DCs (10^4) (black bars), in the presence of IL-2 (20 U/ml) or IL-10 (50 ng/ml) as indicated. After 14 days supernatants were harvested and tested for IgA, IgG and IgM production using specific ELISA's. Data shown are the mean +SD of fivefold cultures and are representative of 3 independent experiments.

Co-culture of dendritic cells from IgAN patients with naïve B cells results in a reduced increase in IgA production

In order to compare the capability of DCs from IgAN patients with DCs from healthy volunteers in the induction of IgA switch in naïve B cells, monocytes were isolated from IgAN patients (n=12) and healthy volunteers (n=12).

Controls and IgAN patients were always tested in parallel with the same source of B cells and the same L-CD40L cells. Co-culture in the presence of IL-10 resulted in less IgA production induced by DCs from IgAN patients as compared to DCs from healthy control persons (5.34 µg/ml versus 2.34 µg/ml; p=0.026) (Figure 5A). No difference between patients and controls was observed when cocultures were performed in the presence of IL-2. To determine the specificity for the IgA regulation, also IgG and IgM were measured in the same supernatants. In all conditions, there were no differences between the IgAN patient group and the control persons with respect to IgG and IgM production (Figure 5B).

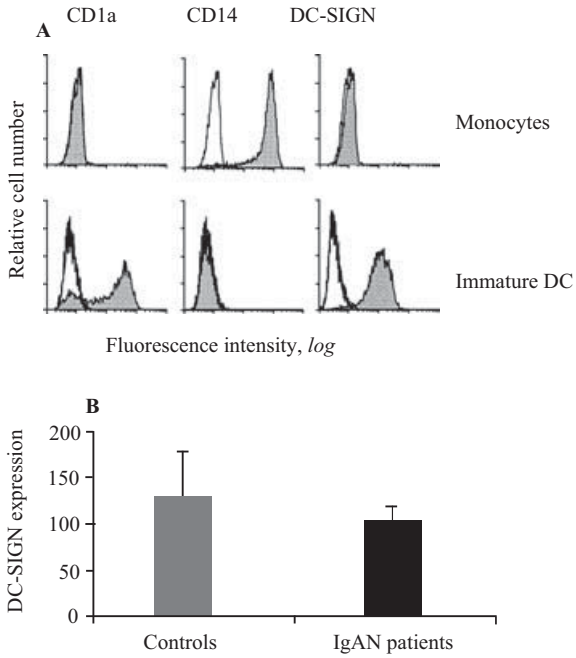

Figure 3. Analysis of CD1a, CD14 and DC-SIGN expression on monocytes and immature dendritic cells from controls and patients with IgAN. *(***A**) Surface expression of CD1a, CD14 and DC-SIGN on monocytes (upper panel) and monocyte derived DCs (lower panel) was determined using FACS analysis. Specific staining is represented by the filled histograms and control staining by the open histograms. Shown is a representative example of DCs from a control. (**B**) Surface expression of DC-SIGN on DC from control persons and DC from IgAN patients was determined using FACS analysis. Shown are the mean fluorescence intensity (MFI) ±SD of 5 control persons and 5 IgAN patients.

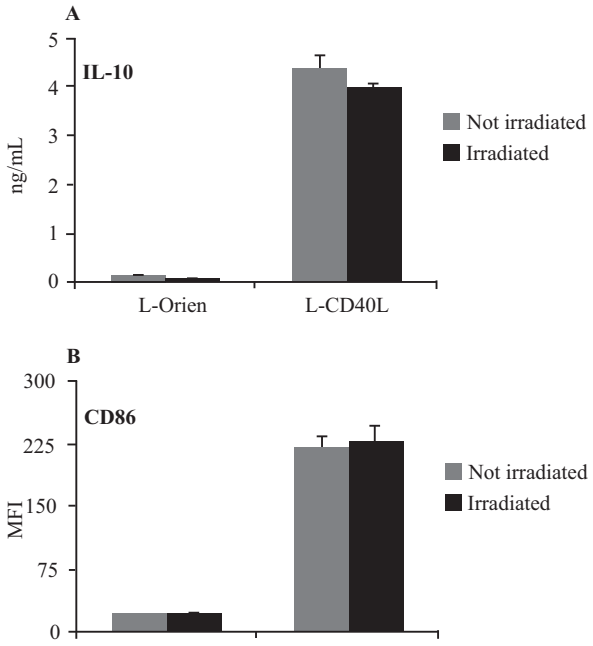

Figure 4. Irradiation of DCs does not influence CD40L induced IL-10 production and CD86 expression. (**A**) DCs ($5x10^4$) were either irradiated (33 Gy) or not irradiated and cultured for 72 hours in the presence of control L cells (left panel) or CD40L-transfected L cells (10^4) (right panel). IL-10 production was measured by specific ELISA. Shown are the mean $\pm$SD of quadriple cultures. (**B**) Surface expression on DCs of CD86 was determined by FACS analysis. Shown is the mean of three experiments.

In our analysis it was clear that there was a large individual variation. Therefore next to the mean absolute production of IgA we calculated the production of IgA induced by the patient-DC as a ratio of the response induced by control-DC in the same experiment. From this analysis it is clear that although the mean ratio of the 12 patients investigated is 0.43 ± 0.32, this ranged between a complete deficiency (ratio<0.05) and a normal response (ratio 1.05) (Figure 6).

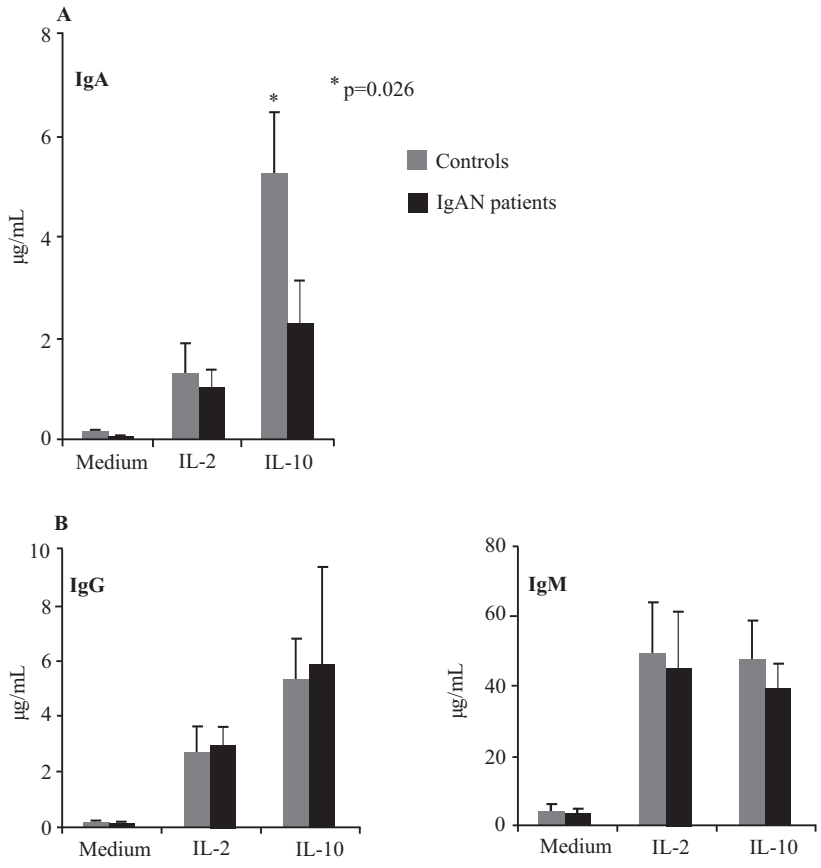

Figure 5. DC from IgAN patients induce less net IgA production compared with controls. (**A**) Highly purified IgD^+ B cells (10^4) were cultured with 75 Gy irradiated CD40L transfected L cells ($0.25x10^4$) in the presence of DCs from controls (n=12) or in the presence of DCs (10^4) from patients with IgAN (n=12), in the presence of IL-2 (20 U/ml) or IL-10 (50 ng/ml) as indicated. After 14 days supernatants were harvested and tested for IgA. Data shown are the mean ±SEM of fivefold cultures of 3 independent experiments. (**B**) IgG and IgM production was measured using specific ELISA's. Data shown are the mean ±SD of five fold cultures of 3 independent experiments.

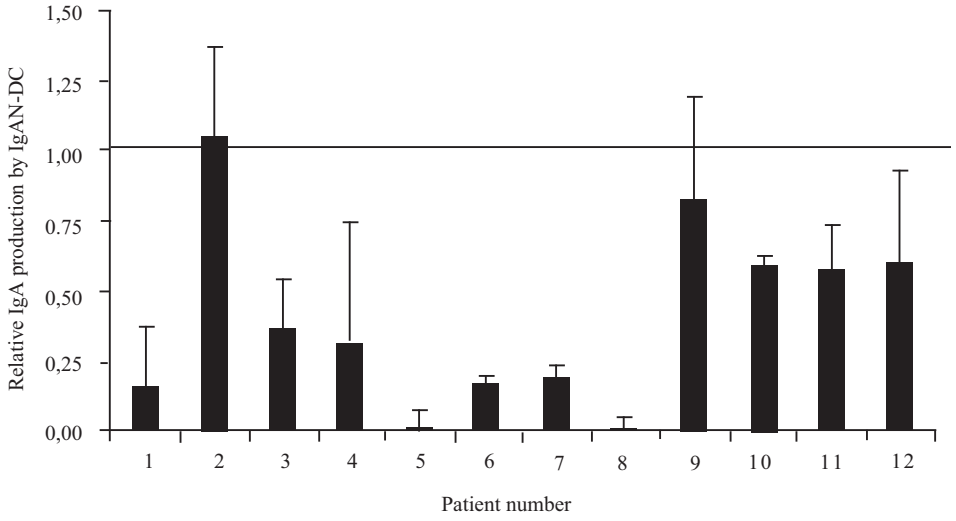

Figure 6. Ratio of IgA production induced by DCs from patients relative to controls. Mean individual IgA production induced by DC from patients with IgAN is divided by the mean IgA production induced by DC from control persons and expressed as a ratio. Shown are the mean +SD of five measurements.

Dendritic cells from IgAN patients induce less IgA_1 and IgA_2 production than DCs from healthy control persons

Next to the measurements of total IgA, also production of IgA subclasses, IgA_1 and IgA_2 were determined. Comparable with total IgA, the addition of DC together with IL-2 and IL-10 to naïve B cells induced both IgA_1 and IgA_2 production. In line with previous results and with the difference in serum concentrations of these subclasses, the production of IgA_1 was much stronger. As observed for total IgA, DCs derived from IgAN patients induced less IgA_1 as well as IgA_2 compared to controls, which was again restricted to the conditions with exogenous IL-10 (Figure 7).

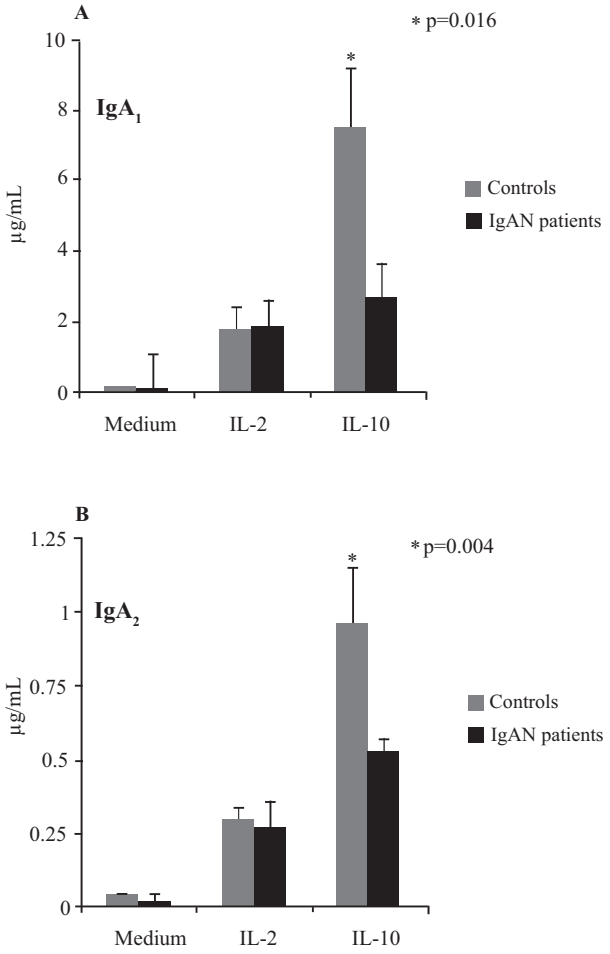

Figure 7. DCs from patients with IgAN induce less IgA_1 and IgA_2. Highly purified IgD^+ B cells (10^4) were cultured with 75 Gy irradiated CD40L transfected L cells ($0.25x10^4$) in the presence of DCs from control persons (grey bars) (n=12) or in the presence of DCs (10^4) from patients with IgAN (black bars) (n=12), in the presence of IL-2 (20 U/ml) or IL-10 (50 ng/ml) as indicated. After 14 days supernatants were harvested and tested for IgA_1 (**A**) and IgA_2 (**B**). Data shown are the mean ±SD of fivefold cultures of 3 independent experiments.

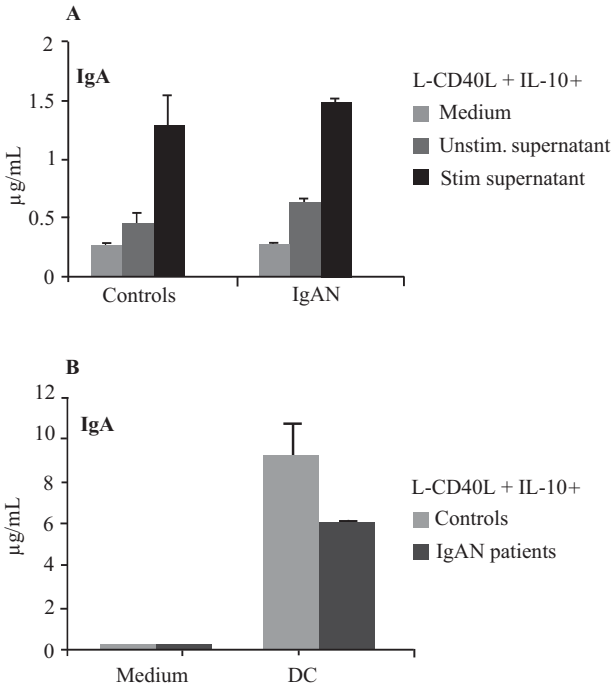

Figure 8. Supernatant of CD40L stimulated DCs can induce IgA switch, but to a lesser extent than DCs. (**A**) DCs ($5x10^5$) of control persons and IgAN patients were cultured in the presence of medium, control L-cells (unstimulated supernatant) or CD40L-transfected L-cells (stimulated supernatant) (10^5) in a total volume of 500 µl. After 72 hours supernatants were harvested and 50 µl of each supernatant was added to naïve B cells (10^4) in the presence of IL-10 (50 ng/ml). After 14 days supernatant was harvested and tested for IgA, using specific ELISA. (**B**) DCs (10^4) from the same control persons and IgAN patients were added to naïve B cells (10^4) in the presence of medium or IL-10 (50 ng/ml). After 14 days supernatant was harvested and tested for IgA, using specific ELISA.

Supernatant from cultured dendritic cells can induce IgA production in the presence of IL-10

In order to investigate whether a soluble factor, produced by DCs, is responsible for the differences in IgA production between the two groups, we generated supernatant from CD40L-stimulated DCs and from DCs cultured without CD40L. After 72 hours supernatant from this culture system was harvested

and added to naïve B cells in the presence of CD40L with or without IL-10. In the absence of exogenous IL-10, DC supernatant could not induce IgA production (data not shown). However in the presence of IL-10, especially supernatant of CD40L-activated DC could augment IgA production. However it should be noted that this effect is small compared to the effect of intact cells and importantly did not show a difference between controls and patients with IgAN (Figure 8A). In the same experiments, irradiated DCs were also investigated using the same B cells demonstrating again a hyporesponse of DC from IgAN patients (Figure 8B).

Discussion

In the present study, using an in vitro model, we show that monocyte-derived DCs of IgAN patients have a reduced capacity to induce IgA_1 and IgA_2 production in naïve B cells compared to DCs from control persons. This difference was seen provided that CD40L and IL-10 were present. In contrast, IL-2 also increased immunoglobulin production, but in this condition there was no difference in the B cell stimulatory capacity of DCs derived from IgAN patients or controls. No differences in IgG and IgM production were measured, independent of the different cytokines that were used. Addition of supernatant from CD40L stimulated DCs to naïve B cells resulted in Ig production, but did not result in a difference between controls and IgAN patients. This suggests that a DC membrane bound factor is responsible for the difference.

IgAN is a disease with a wide variety in clinical presentation and outcome. Some patients have a rapidly progressive renal deterioration leading to renal failure within a short time period, while others have stable kidney function for many years. Eventually about 30-40% of the patients will develop renal failure. It is generally accepted that genetic factors are involved in the pathogenesis of IgAN [34]. Not only familial cases of IgAN have been described, but also a linkage between locus 6q22-23 and IgAN has been described [35;36]. It is tempting to speculate that the variation in IgA

stimulatory capacity (Figure 6) might relate to some of these genetic factors. Alternatively, the in vitro hyporesponsiveness could potentially relate to the severity of IgAN. However in our study there was no correlation between clinical parameters like creatinine clearance or hematuria and the IgA inducing capacity (data not shown).

Vaccination studies have clearly demonstrated that patients with IgAN have a disturbed regulation of the mucosal immune response. Using CTB as a neoantigen we have previously shown that patients with IgAN show a specific IgA hyporesponsiveness, with normal IgG and IgM responses [15]. Importantly, simultaneous systemic administration of another neoantigen did not result in an IgA hyporesponse. These results indicate that the defects observed in IgA production are not generalized, but might be confined to the mucosal compartment. Regulation of IgA production is a complex process dependent on B cells, T cells and antigen presenting cells, but might also be affected by environmental mucosal factors. Previous in vitro studies investigating B cells or T cells of IgAN patients have not clearly pinpointed towards a molecular defect [37-39].

Total IgA production depends on IgA switch by IgD^+ B cells [26] and further proliferation and differentiation of these B cells. Class switch to IgA is a complex event, in which rearrangement of genes has to take place [40-42]. This process is dependent on the interaction between T cells and B cells and is influenced by many other factors. A key role in this process is the interaction between the TNF-R member, CD40 and its ligand [43]. This is demonstrated in the hyper-IgM syndrome in which a genetic alteration of the CD40L gene is responsible for low amounts of serum IgA, IgG and IgE [23;24]. Next to CD40L, IgA production is regulated by cytokines like IL-10 and TGF-β that act directly on B cells. Moreover as also shown in this study, DCs can further increase IgA production. Previous studies using similar IgD^+ tonsillar B cells have demonstrated that DCs augment the isotype switch process as proven by the increase in switch circles [44]. Although our system did not allow a direct investigation of the switch process, it is likely that this contributes to the increased IgA production. The nature of the DC signal promoting IgA production is at present not known. Although there is a B cell stimulating

factor in the supernatant of activated DC, our experiments with supernatant suggest that the difference between patients and controls seems to be caused by a membrane factor. Recently it has been shown that signalling through BAFF, another member of the TNF-R family, can also augment IgA switch in a CD40-independent manner. However, it was shown that BAFF acted in concert with anti-IgM B cell activation, but not in concert with CD40 signalling [45]. Moreover in preliminary experiments we were not able to show BAFF expression on normal monocyte derived DCs (data not shown). Therefore it is not likely that difference in BAFF expression can explain the observed in vitro differences in IgA production.

In the present study we have investigated monocyte-derived DC, and have tried to link the functional capacity of these cells to the observed mucosal hyporesponsiveness. At present it is unclear how these cells relate to mucosal DC populations. It will be a major technical challenge to investigate the functional capacities of mucosal DC directly, especially due to the limited access to mucosal tissues of these patients. However an intruiging observation in our study was that the defective IgA production, induced by DC, was only observed in the presence of IL-10. When DC were combined with IL-2, there were no functional differences between patients and controls. This suggests that there are at least two different mechanisms by which DC can increase IgA production by naive B cells. Until now there are no clues about the underlying molecular mechanisms. Similarly, it is not clear under which conditions in vivo IgA production is under the control of IL-2 or IL-10. Genetic inactivation of IL-10 in mice does not result in major B cell defects or reduced circulating Ig levels [46], whereas IL-2 deficiency does show dramatic reduction of circulating Ig levels [47]. In both cases, the mice develop chronic mucosal inflammation, suggesting a role in mucosal immune regulation [46;48]. However, it seems that expression of IL-10 is more widely distributed and that IL-10 has a more important role as an anti-inflammatory mediator [49;50]. It would be attractive to link the differential response in combination with IL-2 or IL-10, with the observed selective deficiency of a mucosal challenge, as discussed above [15]. Which local mucosal factors contributed to the earlier described IgA hyporespons after vaccination remains

unclear. It certainly needs further research to determine the precise role of DC and IL-10 in the regulation of mucosal IgA production.

In conclusion the current data strongly suggest that DCs of IgAN patients have an impaired capability to enhance IgA production. These data support the results from an earlier study in which mucosal vaccination was accompanied with an IgA hyporesponse [15]. The pathogenetic role of IgA hyporesponsiveness in IgAN could be that an initially inadequate clearance of an antigen leads to a prolonged immune reaction. This prolonged immune reaction could eventually lead to an increased serum IgA titer. Which molecular factors are responsible for the impaired IgA response and whether the impaired capacity to induce IgA switch is restricted to certain subsets of DCs, is still unknown and further studies are needed to resolve these questions.

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