Interleukin 10 Induces B Lymphocytes from IgA-deficient Patients to Secrete IgA

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Abstract

We have previously shown that human B lymphocytes cultured in the CD40 system, composed of an anti-CD40 mAb presented by a CD32-transfected fibroblastic cell line, proliferate but do not secrete antibodies. However, the addition of particles of Staphylococcus aureus Cowan (SAC) induces B cell differentiation even in the absence of exogeneous cytokines (CD40/SAC system). Additionally, B lymphocytes cultured in the CD40 system in the presence of human IL-10, produce IgM, IgG, and IgA, and Ig levels are further increased by SAC. Here, we have studied the capacity of peripheral blood lymphocytes from patients with IgA deficiency (IgA-D) to secrete Igs, particularly IgA after CD40 triggering. Peripheral blood mononuclear cells (PBMNC) from IgA-D patients cultured in the CD40/SAC system produced IgM and IgG, but not IgA. The addition of IL-10 to the cultures, enhanced the production of IgM and IgG and most strikingly induced the production of high amounts of IgA. The addition of IL-10 to PBMNC from IgA-D patients activated through CD40 alone resulted in the production of IgA. Thus, SAC and anti-CD40 mAb stimulate B cells to differentiate into cells secreting IgG and IgM whereas IL-10 plays a central role in inducing B cells from IgA-D patients to differentiate into IgA secreting cells. (J. Clin. Invest. 1994. 94:97–104.) Key words: IgA deficiency • interleukin-10 • B lymphocytes • CD40 triggering • differentiation

Introduction

IgA deficiency, the most common primary immunodeficiency, is known to be a heterogeneous clinical disorder (1). Genetic studies indicate that susceptibility genes located in the major histocompatibility complex class III region may predispose homozygous individuals to this pathology (2, 3). Environmental factors may also play a role in the occurence of IgA deficiency, as several pathologies including viral infections have been found associated with IgA deficiency (1). Although the basic immunologic defect that gives rise to IgA deficiency is unknown, a number of in vitro immunological alterations have been identified. In vitro cell culture studies have revealed that mechanistically IgA deficiency can be classified into three groups: (a) an arrest in the B cell differentiation pathway (4); (b) an increased in suppressor T lymphocyte function (5, 6); and (c) a decrease in helper T lymphocyte function (6, 7). It has been proposed that B cells from IgA-D patients have undergone an isotype switch towards IgA since peripheral B cells from these patients bear surface IgA although they do not differentiate into IgA producing cells (8). Recently we developed B cell culture conditions in which an anti-CD40 mAb is presented by a CD32-transfected fibroblast cell line (CD40 system). This system provides the signals for B cell activation and proliferation, thus allowing us to determine the capacity of cytokines to enhance B cell proliferation and induce Ig synthesis (9, 10). In this context, the addition of IL-10 enhances short term B cell proliferation and more strikingly induces B cells to differentiate into high Ig secreting plasma cells (11). The addition of particles of Staphylococcus aureus Cowan (SAC)[1] to the CD40 system results in considerable secretion of Igs in the absence of isotype switch (CD40/SAC system) (12). Recently a CD40 ligand was identified on activated T cells and its gene subsequently cloned (13, 14). Cells transfected with the gene for CD40 ligand induce B cell activation and proliferation similar to the CD40 system under study in our laboratory (14). The present study is aimed at determining whether different forms of IgA deficiency (acquired and primary) might be related to intrinsic B cell defects or to regulatory T cell abnormalities. In particular, the effects of IL-10 on Ig production by PBMNC or purified B lymphocytes from IgA-D patients was investigated. Here we show that CD40-activated B cells from acquired or primary IgA-D patients can produce IgA when cultured in the presence of IL-10.

Methods

Patients. Selective acquired IgA deficiency was observed in four patients (BEAU, BIG, ORM, and REB) among a group of 335 adult renal transplant recipients. The clinical and immunologic parameters of these patients have been reported previously (15). These patients had normal Ig serum levels during maintenance hemodialysis and before grafting (0.8–4 g/l). An abrupt decline in IgA serum levels occurred 6 wk after transplantation concurrently with a transient hepatitis. Hepatitis occurred in one chronic hepatitis B (Hbs) carrier patient and in three others with active cytomegalovirus (CMV) disease. Selective IgA deficiency persisted over succeeding years. Immunosuppressive therapy was conventional (anti-thymocyte globulin, azathioprine and low dose corticosteroids).

Two adult patients who had primary IgA deficiency were included in this study: RAF, dialyzed but not transplanted, and BAC who had

1. *Abbreviations used in this paper:* CVI, common variable immunodeficiency; PBMNC, peripheral blood mononuclear cells; SAC, *Staphylococcus aureus* Cowan.

Table I. Clinical Data

Patients	Initial disease	Outcome	Sex	Age*
				yr
Acquired IgA-D patients				
BEAU	Reflux	Chronic rejection Tx favorable	F	40
BIG	Crescentic GN	Chronic rejection On dialysis	M	37
ORM	Membraneous GN	Arterial thrombosis On dialysis	F	43
REB	GN	Tx favorable	M	61
Primary IgA-D patients				
Adults				
RAF	On dialysis Not Tx		M	81
BAC	Tx Immunosuppressed	Tx favorable	F	45
Children				
TON	pneumonia	IvIg therapy	M	8
ZAR	bronchiectasis	Immunostimulant[‡]	M	4
MAR	upper respiratory tract infections	"	M	12
MARC	bronchiectasis	"	M	7
FAS	upper respiratory tract infections	"	M	14
PAV	upper respiratory tract infections	"	M	5
JUS	pneumonia	IvIg therapy	F	5

GN, glomerulonephritis; *Tx*, transplanted. * At the date of the experiments. [‡] Immunostimulants used were Biostim® or Ribomunyl®.

been successfully transplanted and was under immunosuppressive therapy.

In addition, seven children between 4 and 14 years of age (one female and six male), referred in the past two years to the department of Pediatrics for immunologic evaluation for recurrent infections had low serum IgA. Children who had recurrent upper respiratory tract infections were treated by antibiotics and immunostimulatory therapy. Two of the children had I.V.Ig therapy treatment after pneumonia (TON and JUS). All subjects were Caucasian.

Six additional unrelated Caucasian adults (three male and three female) served as controls. Table I shows the significant clinical data available for the two groups of IgA-D patients.

Reagents. The anti-CD40 monoclonal antibody mAb89 was produced in our laboratory (16). The CD32/FCγRII transfected Ltk- cell line (CD32 L cells) was described earlier (17). Cell phenotypes were determined using anti-CD3, -CD4, -CD8, -CD19, -CD56 (anti-NK cells), and -CD14 (anti-monocytes) FITC conjugated mAbs originating from Becton Dickinson (Mountain View, CA). Purified rhIL-2 (Amgen Biologicals, Thousand Oaks, CA) (3 × 10⁶ U/ml) and rhIL-10 (Schering-Plough Research Institute Dardilly, France) (1 × 10⁷ U/ml) were respectively used at 20 U/ml and 100 ng/ml.

Isolation of cells and culture conditions. Peripheral blood mononuclear cells (PBMNC) were isolated using a standard Ficoll (d = 1,077 g/ml) gradient method. Peripheral blood B cells were first enriched in the E⁻ fraction and submitted to anti-CD2, anti-CD3, and anti-CD14 mAbs negative selection with magnetic beads coated with anti-mouse IgG (Dynabeads; Dynal, Oslo, Norway) as described previously (18). Finally, unseparated B cells were sorted using a FACSstar^plus flow-cytometer (Becton Dickinson) after CD19-FITC labeling. For Ig production, formalinized particles of *S. aureus* strain Cowan I (SAC) pur-

chased as Pansorbin from Calbiochem-Behring Corp. (La Jolla, CA) was used as a polyclonal activator at the final concentration of 0.005% (vol/vol), either alone or in combination with the CD40 system. The CD40 system is composed of 5 × 10³ irradiated (7,000 rad) CD32 L cells and 0.5 μg/ml of anti-CD40 mAb89, where 5 × 10⁴ PBMNC or purified B cells are cultured in a final volume of 200 μl. Supernatants were harvested after 10 d and Ig levels were determined by ELISA (19). Cultures were carried out in modified Iscove's medium as detailed previously (19).

Cytokine production. 10⁶ PBMNC of patients and normal donors were incubated in 1 ml culture medium with PHA (1 μg/ml) and SAC and 20 U/ml of IL-2 for 48 h. Supernatants were harvested and IL-10 and IFNγ contents were determined by specific ELISAs described in detail elsewhere (20, 21).

Results

Phenotyping of PBMNC from IgA-D patients. As shown in Table II, the proportion of B cells (stained by CD19 mAb) within the mononuclear cell population was low for all the acquired IgA-D patients (BEAU, BIG, REB, and ORM) as well as the adult primary IgA-D patient RAF. Furthermore, the CD4 compartment was reduced for patients BEAU, BIG, REB, and BAC. All seven children with primary IgA deficiency had normal proportions of B and T lymphocytes when compared with age matched controls. MARC and PAV had reduced percentages of CD4⁺ T cells. Serum IgA levels (Table III) were below 0.08 g/l for all patients except BIG who had low but detectable

Table II. Phenotype of Freshly Isolated PBMNC

	CD3	CD4	CD8	CD19	CD56	CD14
			%*			
Adults						
Acquired IgA-D patients						
BEAU	38	15	24	2	2	6
BIG	30	15	10	1	15	2
ORM	60	35	25	3	15	3
REB	45	10	35	1	10	2
Primary IgA-D patients						
RAF	60	35	20	4	25	3
BAC	40	15	20	10	3	3
Controls (n = 6)	56–82	28–49	9–25	7–16	ND	9–17
Children						
Primary IgA-D patients						
TON	70	45	25	13	15	15
ZAR	60	40	20	15	5	5
MAR	65	45	25	8	20	15
MARC	76	28	43	10	15	15
FAS	65	41	24	11	ND	ND
PAV	51	25	38	20	32	5
JUS	71	41	26	13	17	5

* Percent of cells positively stained with the mAbs after isolation on density gradient (d = 1.077).

serum IgA levels (0.16 g/l). IgM and IgG levels were within normal range except for patient FAS who had elevated total IgG. The proportion of IgG subclasses varied greatly within this group. Acquired IgA-D patients had lower IgG2 and IgG4 levels whereas within the group of primary IgA-D children, MAR and FAS had increased IgG3 levels compared to normal range reference values (22).

Ig synthesis by PBMNC from IgA-D patients. Purified tonsillar B cells which produce low amounts of Igs when cultured in the CD40 system secrete considerable levels of Igs without isotype switch when particles of SAC are supplemented (12). Thus, to maximize B cell differentiation we tested the production of Igs by PBMNC cultured in the CD40 system together with SAC. As shown in Table IV, PBMNC from controls produced IgG (14.5 μg/ml), IgM (19 μg/ml) and IgA (5.2 μg/ml) when cultured under these conditions (mean obtained with six samples of PBMNC from normal donors). PBMNC from controls cultured with either SAC or the CD40 system alone produced between 0.1 and 0.7 μg/ml of the different isotypes (not shown). PBMNC from IgA-D patients cultured in the CD40 system together with SAC secreted IgG (mean of 2.3 and 3 μg/ml for acquired IgA-D and primary IgA-D, respectively) and IgM (mean of 2 and 5.3 μg/ml for acquired IgA-D and primary IgA-D, respectively). The mean production of IgG was around 4–5-fold less than that of controls while that of IgM was 4–10-fold lower than that of controls. No detectable levels of IgA (< 0.01 μg/ml) could be found in supernatants of IgA-D PBMNC, whereas normal donors produced as much as 5.2 μg/ml IgA. Thus mononuclear cells from IgA-D patients are unable to produce IgA while they secrete quite significant levels of IgM and IgG after CD40/SAC triggering. These data

provide in vitro confirmation of the diagnosis of IgA deficiency (Table III).

IL-10 induces IgA synthesis by PBMNC from IgA-D patients. As IL-10 has earlier been found to induce CD40-activated naive B cells to produce small amounts of IgA (12), we explored whether it would induce B cells from patients with IgA-D to produce IgA. Thus, PBMNC from the 13 IgA-D patients were cultured in the CD40/SAC system and in the presence of IL-10. As shown in Fig. 1, PBMNC from IgA-D patients activated by anti-CD40 mAb and SAC secreted IgA when IL-10 was added to cultures. The levels of IgA obtained varied from one patient to another. The quantities of IgA produced by the PBMNC from acquired IgA-D patients (Fig. 1 A) ranged from 0.3 to 1.6 μg/ml of IgA. Even more strikingly, PBMNC from primary IgA-D patients (Fig. 1 B) secreted up to 7.9 μg/ml IgA in response to IL-10. While the mean IgA levels obtained with IgA-D PBMNC in response to IL-10 (mean±SD = 3±2.6 μg/ml IgA, n = 13 IgA-D patients) was lower than of controls (Fig. 1 C) (mean±SD = 10.8±2.4 μg/ml IgA, n = 6 controls), it is noteworthy that some IgA-D patients produced as much IgA as controls.

As can be seen in Fig. 2, the effect of IL-10 was not restricted to IgA isotype. Addition of IL-10 also increased IgG (2–5-fold) and IgM (3–10-fold) productions by activated PBMNC from normal donors and IgA-D patients. Cells from primary IgA-D patients produced almost as much IgG and IgM as controls, while those from acquired IgA-D produced less Ig. This may be related to the lower B cell numbers in acquired IgA-D patients (Table II). As shown in Fig. 3, IL-10 increased IgA secretion by PBMNC of both IgA-D patients (ZAR and MAR) and controls in a dose-dependent fashion. Significant IgA syn-

Table III. Seric Ig Levels

Donors	IgG	IgA	IgM	IgG1	IgG2	IgG3	IgG4
Adults				*g/l*			
Group I: acquired IgA deficient patients*							
BEAU	12	< 0.08	2.77	6.5	0.2	0.26	0.01
BIG	15.5	0.16	1	5.85	0.35	0.15	0.01
ORM	15.9	< 0.08	1.34	13	0.1	0.75	0.05
REB	6.9	< 0.08	0.37	4.8	0.3	0.15	0.04
Group II: inherited IgA deficient patients*							
RAF	18	< 0.08	2.44				
BAC	12.2	< 0.08	3.1				
Reference values							
normal controls	8–16	0.8–4	1–3	3.2–12	1.2–3.5	0.2–0.8	0.2–0.7
Children							
Group II: inherited IgA deficient patients*							
TON	15.9	< 0.08	1.58	9.6	1.3	0.38	0.52
ZAR	18.3	< 0.08	1.92	12	2.2	0.19	0.2
MAR	11.1	< 0.08	0.95	7.2	3.2	1	0.4
MARC	11	< 0.08	0.82	9.4	1.8	0.4	0.28
FAS	23.4	< 0.08	2.29	18.8	3.2	2.14	< 0.02
PAV	16.3	< 0.08	1.01	12.5	0.9	0.3	0.17
JUS	13.6	< 0.08	1.75	10.3	3.1	0.27	0.21
Reference values							
normal controls (age in years)							
4–8	5–14	0.5–2.2	0.5–2	3.8–12	0.4–3	0.2–0.6	0.1–0.8
8–10	"	"	"	4.9–11	0.5–3	0.2–0.6	0.2–0.7
10–13	"	"	"	3.6–11	0.5–3	0.2–0.6	0.3–0.7

* Mean of 3–5 determinations over 2 yr preceeding the experiments. Seric Ig contents were determined by nephelometry.

thesis could be detected with 10 ng/ml of IL-10 and maximum IgA synthesis was obtained with 100 ng/ml of IL-10.

IL-10 directly acts on B cells from IgA-D patients. Whenever the sample size allowed it, to determine whether IL-10 directly acts on B lymphocytes, these were purified from blood mononuclear cells by FACS-sorting CD19 positive cells (95% purity). B cells were then cultured with or without IL-10 in the CD40/SAC system. As shown in Fig. 4, highly purified B cells from two children with primary IgA deficiency (ZAR and JUS) produced IgA in response to IL-10. As observed earlier with IgA-D PBMNC, IL-10 enhanced the production of IgG and IgM by purified B cells of these IgA-D patients (Fig. 4). In control experiments, incubation of patient PBMNC with the anti-CD19 mAb alone did not alter the subsequent Ig production (data not shown).

To discriminate the relative contribution between SAC, anti-CD40 mAb and IL-10 on Ig synthesis, we cultured PBMNC from primary IgA-D patients (TON, ZAR, and MAR) with SAC in the presence or absence of IL-10, or anti-CD40 mAb plus SAC without or with IL-10. As shown in Table V, PBMNC activated with SAC did not result in IgA production even in the presence of IL-10. Also, triggering of PBMNC by anti-CD40 mAb alone was not sufficient to induce IgA synthesis. However, addition of IL-10 to CD40-triggered PBMNC resulted

in the production of IgA. Finally, addition of SAC augmented the effect of IL-10 on Ig synthesis when PBMNC were cultured in the CD40 system (6–19-fold increased IgA levels with anti-CD40 mAb/SAC plus IL-10 compared with anti-CD40 mAb plus IL-10). Thus, anti-CD40 and SAC stimulated B cells to differentiate into cells secreting IgG and IgM (Table IV), whereas IL-10 was instrumental in inducing B cells of IgA-D patients to differentiate into IgA secreting cells.

PBMNC from IgA-D patients produce IL-10. Because IL-10 efficiently restores the defective IgA production by the B cells of patients with IgA-D, we explored the possibility that this pathology may be associated with altered IL-10 production. However, as shown in Fig. 5, PBMNC from IgA-D patients activated with SAC, PHA and IL-2 (21) produced significant levels of IL-10, ranging from 0.5–1.8 ng/ml (mean±SD = 1.1±0.5). Note, this amount of IL-10 is significantly lower (P = 0.0019, Mann and Withney U test) than that obtained with normal control PBMNC (mean±SD = 2.8±0.5). At the end of cultures, levels of IL-10 produced by PBMNC in response to anti-CD40 mAb/SAC activation were in the order of 500 pg/ml with no significant differences noted between IgA-D patients and controls (not shown).

Next, we explored whether the reduced production of IL-10 by cells from IgA-D patients could be due to increased

Table IV. Ig Production by PBMNC from Normal Donors and
IgA-D Patients in Response to SAC and Anti-CD40

	IgG	IgA	IgM
		μg/ml	
Controls			
1	15.3±1.9	7.3±1.5	19.7±2
2	19.5±2	6±1	13.8±0.6
3	3.6±0.5	4±0.5	19±1.6
4	9.6±2	4.5±0.5	7.1±1
5	28.8±2.1	5.2±0.9	19.6±2
6	9.7±1.9	4±0.3	35.6±4
Mean±SD	14.5±8	5.2±1.2	19.0±8.6
Acquired IgA-D patients			
BEAU	3.3±0.3	< 0.01	3.7±0.8
BIG	0.6±0.1	< 0.01	0.6±0.1
REB	3.7±0.2	< 0.01	1.6±0.1
ORM	1.8±0.6	< 0.01	2.2±0.5
Mean±SD	2.3±1.2		2.0±1.1
Primary IgA-D patients			
RAF	1.4±0.3	< 0.01	14±1.5
BAC	6±0.9	< 0.01	3.3±0.1
TON	4.2±0.2	< 0.01	2.1±0.4
ZAR	11.5±1.5	< 0.01	13.6±1.7
MAR	1.7±0.4	< 0.01	8.4±1
MARC	1.5±0.3	< 0.01	2.7±0.5
FAS	0.2±0.03	< 0.01	2±0.1
PAV	0.13±0.02	< 0.01	1±0.2
JUS	0.2±0.03	< 0.01	0.9±0.05
Mean±SD	3.0±3.5		5.3±5

5×10^4 PBMNC were cultured on 5×10^3 irradiated CDw32 L cells with 0.5 μg/ml anti-CD40 mAb (mAb89) and SAC. Ig levels (μg/ml) were determined after a 10-d culture period. Data are expressed as mean±SD values of triplicate cultures.

Figure 1. IL-10 induces IgA production by PBMNC from IgA-D patients. 5×10^4 PBMNC from acquired or primary IgA-D patients and normal donors were cultured on 5×10^3 irradiated CDw32 L cells with 0.5 μg/ml mAb89 and SAC (0.005% vol/vol) with (■) or without (□) 100 ng/ml IL-10. Culture supernatants were harvested after 10 d in culture. IgA levels (μg/ml) are expressed as mean±SD values of triplicate cultures.

synthesis of IFNγ, an antagonist of IL-10 production (23). However, following activation, PBMNC from IgA-D patients produced levels of IFNγ comparable with those obtained with controls ($P > 0.05$) (Fig. 5). IL-2 and IL-4 levels were also comparable in both groups (data not shown) thus excluding a clear-cut disequilibrium in the production of those cytokines.

Discussion

In the present study, we have induced B lymphocytes from IgA-D patients to secrete IgA in response to a combination of three well known B cell activators: anti-CD40 antibody, particles of SAC and interleukin 10. This combination of activators was particularly efficient as cells from all IgA-D patients tested were induced to produce IgA, irrespective of whether patients had acquired or primary IgA deficiency. However, cells from primary IgA-D patients produced more IgA than those from acquired IgA-D patients. In this context, cells from acquired IgA-D patients also produced less IgM and IgG than those from primary IgA-D patients or controls. This observation correlates with the fact that the acquired IgA-D patients had very low numbers of circulating B lymphocytes, which may be related to their undergoing dialysis over an extensive period of time. Within the group of primary IgA-D patients, 3/9 produced low
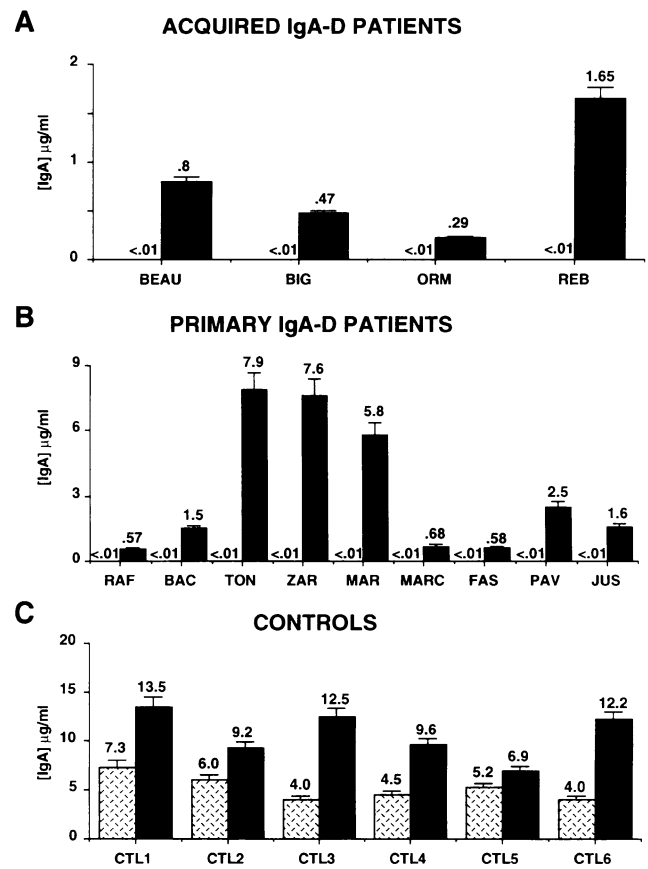
levels of IgA in response to IL-10 (reaching 6% of controls), while the majority of primary IgA-D cells (6/9) produced nearly normal amounts of IgA in this system (20–80% of controls). None of the patients included in this study were asymptomatic, thus further studies are required to assess whether the levels of IgA obtained in this system are comparable in asymptomatic IgA-D cells with those obtained with IgA-D patients suffering of recurrent infections.

Because of limited sample size, most of our study was performed with whole blood mononuclear cells. However, we were able to purify B lymphocytes from two primary IgA-D patients (ZAR and JUS) which IgA secretion by PBMNC reached nearly normal amounts in response to IL-10. Highly purified blood B cells (95% pure) from those primary IgA-D patients were also induced to secrete levels of IgA comparable to those of normal donors. It is unlikely that the remaining 5% contaminant non-B cells may play an indirect role in the effect of IL-10 on IgA synthesis. Also, we have reported previously that sIgD+ naive B cells (> 98% pure) isolated from tonsils secreted low but reproducible levels of IgA in response to IL-10 when activated through CD40 (12). However, it is not known whether CD40 triggering and/or addition of IL-10 enhances the production of TGFβ which has been reported to induce switch at a molecular
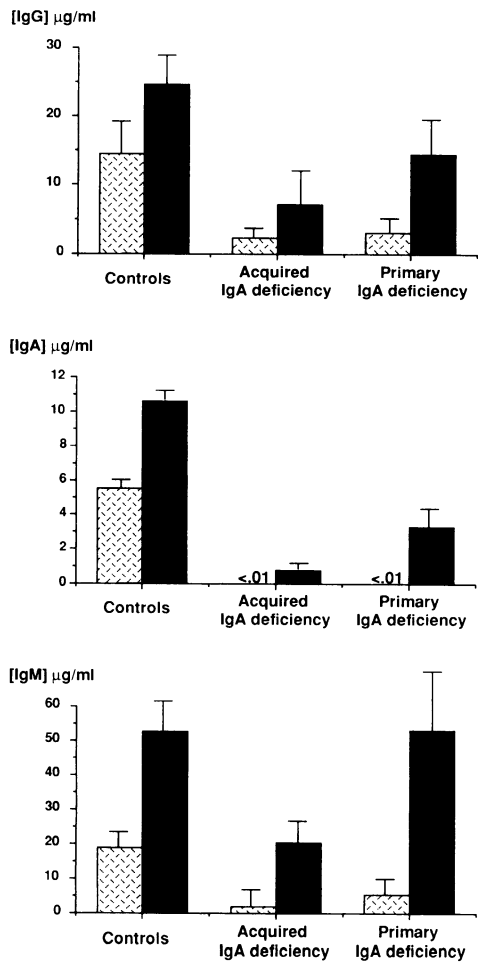
[IgG] μg/ml

[IgA] μg/ml

[IgM] μg/ml

[Ig] μg/ml

IgA

IgG

IgM

Figure 4. IL-10 induces B cells from IgA-D patients to produce IgA. 5 × 10⁴ anti-CD19 FACS-sorted B cells (95% pure) from two primary IgA-D patients (ZAR, ■; and JUS, □) were cultured on 5 × 10³ irradiated CDw32 L cells with 0.5 μg/ml mAb89 and SAC (0.005% vol/vol) with or without 100 ng/ml IL-10. Culture supernatants were harvested after 10 d of culture and Ig levels (μg/ml) are expressed as mean±SD values of triplicate cultures.

Figure 2. IL-10 enhances IgG, IgA, and IgM in PBMNC from both controls and IgA-D patients. 5 × 10⁴ PBMNC from acquired or primary IgA-D patients and normal donors were cultured on 5 × 10³ irradiated CDw32 L cells with 0.5 μg/ml mAb89 and SAC (0.005% vol/vol) with (■) or without (□) 100 ng/ml IL-10. Culture supernatants were harvested after 10 d of culture and Ig levels (μg/ml) were measured by ELISA. Results are expressed as mean±SD of experiments obtained with normal donors PBMNC (n = 6), PBMNC from acquired IgA-D (n = 4), and primary IgA-D patients (n = 9).

level (24). Nevertheless, the combination of the three activators (anti-CD40 mAb/SAC/IL-10) specifically targets the B cells within the mononuclear cell preparation. The combination of anti-CD40 mAb and IL-10 appeared to be the minimal combination to overcome the differentiation arrest of IgA-committed cells in these patients as shown by low levels of IgA in supernatants of cells stimulated with these two signals. The addition of SAC with these two signals essentially results in an amplification of the Ig secretion. It is important to note that a combination of anti-CD40 mAb and SAC permits normal B cells to produce large amounts of IgM, IgG and IgA, and IgA-D B cells to produce only IgM and IgG but not IgA. Taken together, these results indicate that the addition of IL-10 is a crucial event for the release of the IgA blockade in these patients.

The capacity of CD40 triggering and IL-10 to restore in

[IgA] μg/ml

control

Primary
IgA-D patients
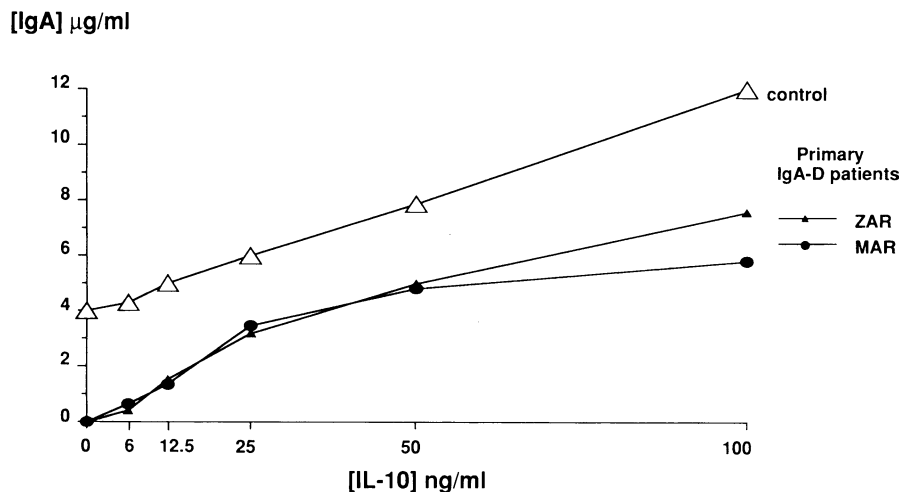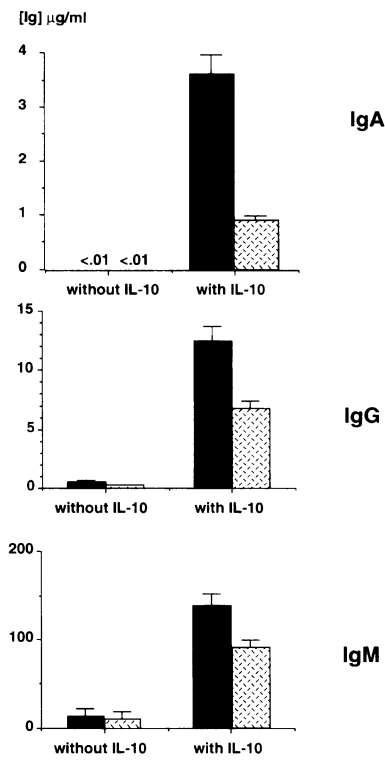
▲ ZAR
● MAR

[IL-10] ng/ml

Figure 3. IL-10 induces dose-dependent IgA synthesis. 5 × 10⁴ PBMNC from primary IgA-D patients (ZAR and MAR) and normal donors (mean of data obtained with 6 controls) were cultured on 5 × 10³ irradiated CDw32 L cells with 0.5 μg/ml mAb89 and SAC (0.005% vol/vol) with or without increasing concentrations of IL-10. Culture supernatants were harvested after 10 d of culture and IgA levels (μg/ml) are expressed as mean±SD values of triplicate cultures.

Table V. Respective Contribution of SAC, Anti-CD40 and IL-10 on Ig Synthesis by PBMNC from IgA-D Patients

Patients	Anti-CD40	SAC	IgG		IgA		IgM	
			−	IL-10	−	IL-10	−	IL-10
					$\mu g/ml$			
TON								
	−	+	.13	.15	< .01	< .01	.05	.05
	+	−	.2	.5	< .01	.2	.1	5.1
	+	+	4.2	30.5	< .01	2.4	2.1	65.2
ZAR								
	+	−	.4	10.2	< .01	1.3	0.7	49.4
	+	+	11.5	28.7	< .01	7.5	13.6	104.8
MAR								
	+	−	.15	4.1	< .01	0.3	0.1	5.3
	+	+	1.7	8.9	< .01	5.8	8.4	112.5

5×10^4 PBMNC were cultured with or without SAC, in the presence or absence of 5×10^3 irradiated CDw32 L cells and 0.5 $\mu g/ml$ anti-CD40 mAb (mAb89) and with or without 100 ng/ml IL-10. Ig levels ($\mu g/ml$) were determined after a 10-d culture period. Data are expressed as mean±SD of triplicate cultures.

vitro Ig production is further illustrated by recent studies on B cells from patients with common variable immunodeficiency (CVI). We and other groups have reported that B cells from most CVI patients can be induced to secrete isotypes such as IgG and IgA in the presence of anti-CD40 mAb and IL-10 (25–27) or IgE in response to anti-CD40 mAb and IL-4 (27). Both pathologies, CVI and IgA-D are heterogeneous groups of disorders characterized by defective antibody production. It has been suggested that IgA deficiency and CVI may represent the extremes of the spectrum of a common B cell defect, thus it will

Figure 5. PBMNC from IgA-D patients produce low levels of IL-10 (■) and normal levels of IFNγ (▨). 10^6 PBMNC from IgA deficient patients were incubated with SAC (0.005% vol/vol), 1 $\mu g/ml$ PHA and 20 IU/ml IL-2. Supernatants were harvested after 48 h, hIL-10 and IFNγ contents were measured by specific ELISAs. Results are expressed as mean±SD values of triplicate cultures. A Mann and Withney U test was used to compare the levels of IL-10 and IFNγ obtained in supernatants of activated PBMNC from IgA-D patients with those obtained from normal donors. The level of significance of the statistical analysis was < 0.05.

be of interest to further dissect the contribution of CD40 and cytokines in the release of a given B cell defect. Furthermore, the capacity or the failure of those signals to restore an effective antibody synthesis, will probably permit us to better identify subgroups of patients within the groups of IgA-D and CVI.

Earlier studies have shown that naive sIgD$^+$ human B lymphocytes produce low amounts of IgA in response to IL-10 and that TGFβ strongly enhances this effect (12). Furthermore, treatment of mice with anti–IL-10 antibodies results in a strong decrease of circulating IgM and IgA (28). It was thus tempting to hypothesize that IgA-D patients may suffer either from a deficit of IL-10 production or from the presence of an IL-10 antagonist. However, mononuclear cells from IgA-D patients were found to produce IL-10 after optimal activation with SAC and PHA, although the levels were slightly lower than those of normal controls. Also, the production of IFNγ which has been shown to antagonize the production of IL-10 (23) was comparable with that obtained with normal donor cells. Levels of IL-10 synthesis induced in SAC and anti-CD40 mAb cultures were low but comparable with normal individuals and IgA-D patients. The fact that a number of cytokines were present in supernatants of activated cells did not allow us to determine whether the IL-10 of IgA-D patients was biologically active. As neutralizing circulating antibodies against IL1α (29), IL-2 (30), IL-6 (31), and IFNγ (32) have been described, the presence of anti-IL10 antibodies was investigated. While anti–IL-10 antibodies could be found in some rare individuals (C. Caux-Ménétrier and F. Brière, manuscript in preparation), they could not be detected in the serum of the 13 IgA-D patients tested. Our future studies will aim at determining whether primary IgA-D patients display functional IL-10 or whether they have an IL-10 antagonist such as soluble IL-10 receptor. This would appear particularly relevant for IgA deficiency associated with IgG1 and IgG3 deficiencies (33) since we recently found that IL-10 induces naive sIgD$^+$ B cells to specifically produce these IgG subclasses (34). Alternatively, these patients may have a blockade in IL-10 production at the physiological sites of B cell differentiation into plasma cells, namely the bone marrow and/or the mucosal lamina propria.
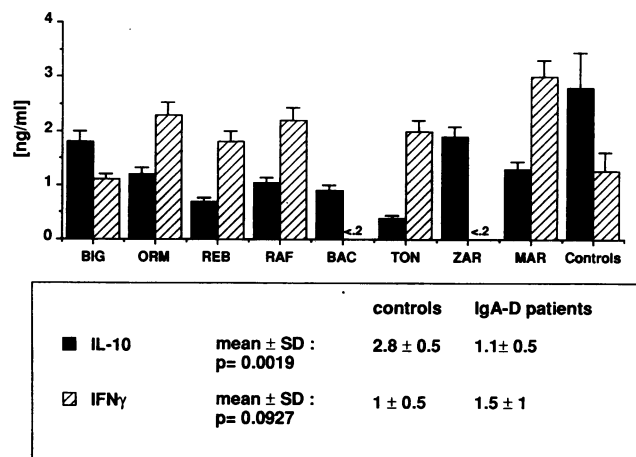
The present study provides a framework for an understanding of the defect underlying the heterogeneous nature of IgA deficiency. In particular, unraveling the molecular mechanisms controlling IL-10–induced differentiation of normal B cells may eventually permit us to understand the basis for the differentiation blockade of IgA-committed B cells in IgA-D patients. In this context, the possibility of a defective CD40 ligand was ruled out, as activated T cells of IgA-D patients express the CD40 ligand which bound a CD40-Fc fusion protein (data not shown). This was indeed expected because patients with altered CD40 ligand are unable to mount isotype switch (35–38).

In conclusion, the present study indicates that the combination of anti-CD40 mAb, SAC and IL-10 induces B cells from IgA-D patients to produce considerable amounts of IgA. Thus, IL-10 appears to be critical in releasing the differentiation blockade of IgA-committed B cells.

Acknowledgments

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