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Increased proportion of responders to a murine anti-CD3 monoclonal antibody of the IgG1 class in patients with systemic lupus erythematosus (SLE)

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SUMMARY

A group of Venezuelan patients with SLE showed an increased proportion of responders to Leu-4, an anti-CD3 MoAb of the IgG1 class, compared with ethnically matched non-SLE patients and healthy controls. The rate of proliferative responses or IL-2 production induced by MoAb Leu-4, and the helper effect of macrophages from Leu-4 responders on T cells from a third-party donor were comparable in patients and controls. No significant differences in the binding of murine IgG1 molecules by macrophages from SLE patients and controls were observed. The proportion of monocytes/macrophages expressing FcγRI was significantly higher in SLE patients. However, the expression of FcRII, the type capable of supporting Leu-4-mediated responses, and of FcγRIII was comparable in monocytes from SLE patients and controls. Our results suggest that Venezuelan patients with SLE may have a genetic predisposition for the expression of the phenotypic variant of FcγRII capable of binding murine IgG1 molecules.

Keywords CD3 activation monocyte function Fc receptors immunoglobulin binding systemic lupus erythematosus

INTRODUCTION

MoAbs specific for the CD3 antigen complex can trigger proliferative responses in human T lymphocytes [1]. Except in very rare cases [2], anti-CD3 MoAbs of the IgG2a class induce T cell proliferation in all normal individuals [1]. On the other hand, only approximately 70% of normal subjects respond to anti-CD3 MoAb of the IgG1 class [3]. The ability to respond to this type of antibody depends on the presence of Fc receptors (FcγR) in accessory cells capable of binding murine IgG1 class molecules [4]. The binding of anti-CD3 IgG1 molecules by these receptors induces cross-linking of CD3 complexes on T cell membranes, which triggers a cascade of subcellular signals leading to full activation and proliferation.

We have recently shown enhanced CD3-mediated responses after stimulation with OKT3, an anti-CD3 MoAb of the IgG2a subclass, in a group of patients with SLE [5]. In that report, all individuals tested were responsive to OKT3 stimulation. In the present study we show that the degree of Leu-4-driven proliferative response and IL-2 production was comparable in Leu-4 responders in patients and controls. However, 82% of SLE patients compared with 62% of non-SLE patients or healthy controls were responsive to stimulation with Leu-4, an anti-CD3 MoAb of the IgG1 class. Monocytes/macrophages from SLE patients showed a helper effect for Leu-4-mediated responses that was comparable to normal. Flow cytometric analysis with MoAbs specific for the three known human FcγR revealed increased numbers of macrophages expressing FcγRI, but not FcγRII or FcγRIII on macrophages from SLE patients. FcγRII have the phenotypic variant capable of binding IgG1 murine MoAbs and conferring the Leu-4 response status [4]. Our results suggest augmented expression of the type of FcγRII with affinity for murine IgG1 molecules in macrophages from Hispanic patients with SLE. This finding may explain the higher proportion of Leu-4 responders in our patient population.

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PATIENTS AND METHODS

Patients

The diagnosis of SLE was established according to the 1982 revised American Rheumatism Association criteria [6]. Forty-five Hispanic patients, 41 female, four male, ages 18–42 years, were classified as active or inactive as proposed by Urowitz *et al.* [7]. Most patients were active ($n = 35$) as defined by the presence of two or more activity criteria [7] and were receiving treatment

with oral prednisone (10–25 mg daily). The average number of clinical activity criteria was 4·1 ± 0·17 (mean ± s.e.m., $n = 35$). Nine patients were also receiving monthly i.v. pulses of cyclophosphamide. To control for non-specific effects related to autoimmunity or treatment with corticosteroids a group of 37 non-SLE patients with immune-mediated disorders receiving oral daily prednisone (range 10–35 mg) was also studied. In this group there were 34 females and three males, including patients with scleroderma ($n = 13$), active rheumatoid arthritis ($n = 13$), renal transplants ($n = 5$), myasthenia gravis ($n = 3$), autoimmune haemolytic anaemia ($n = 1$), peripheral nerve vasculitis ($n = 1$) and chronic glomerulonephritis ($n = 1$). Healthy controls of the same ethnic background, 46 female and 12 male, ages 18–44 years, were obtained from the blood bank and laboratory personnel.

Reagents

Reagents included Percoll and Ficoll (Pharmacia, Uppsala, Sweden), fetal bovine serum (FBS), RPMI 1640, L-glutamine, penicillin, streptomycin (GIBCO Labs, Grand Island, NY) fluoresceinated goat anti-mouse IgG and PE-labelled murine IgG1 (Becton Dickinson, Mountain View, CA), recombinant IL-2 (rIL-2; Genzyme Corp., Boston, MA), and ^{3}H-thymidine, specific activity 2·0 Ci/mmol (New England Nuclear, Boston, MA).

Monoclonal antibodies

OKT3 and Leu-4 MoAbs were purchased from Ortho Diagnostics (Raritan, NJ) and Becton Dickinson, respectively. Anti-T4 and anti-T8 MoAbs identifying the CD4 and CD8 markers on T cells, Mo1 and rhodamine-labelled Mo2 MoAbs, specific for macrophages were obtained from Coulter Immunology (Coulter Corp., Hieleah, FL). B73.1, an anti-CD16 MoAb identifying FcγRIII, was a kind donation of Dr G. Trinchieri (The Wistar Institute, Philadelphia, PA). MoAbs 32.2 and IV.3 specific for FcγRI and FcγRII respectively were a generous gift from Dr C. L. Anderson (Ohio State University, Columbus, OH).

Cells

Peripheral blood mononuclear cells (PBMC), isolated by Ficoll–Hypaque gradients, were resuspended in complete medium containing RPMI 1640, FBS 10%, L-glutamine and antibiotics. T cells were isolated by passage through nylon wool columns followed by a discontinuous density gradient on Percoll [8]. This preparation contained 93 ± 3% $CD3^+$ and < 1% macrophages (0·45 ± 0·11%, mean ± s.e.m.) as tested by flow cytometry with OKT3 and rhodamine-labelled anti-monocyte Mo2 MoAbs respectively. Macrophage-enriched preparations (containing 81 ± 3% peroxidase + cells) were obtained from PBMC after plastic adherence for 45 min at 37°C, followed by incubation in PBS/EDTA (2·5 mM, pH 7·2) for 30 min at 4°C. Adherent cells were recovered with a rubber policeman. Macrophages were treated with mitomycin C (25 μg/ml) for 30 min at 37°C or irradiated with 30 Gy and washed five times before being added in culture.

Flow cytometry

The proportion of cell subpopulations was determined by indirect or direct immunofluorescence and flow cytometric analysis as follows. PBMC (1×10^6) were incubated with an optimal dilution of the corresponding MoAb for 30 min at 4°C and washed twice in cold PBS plus 1% bovine serum albumin (BSA), and 0·02% sodium azide. After incubation for 30 min at 4°C in the presence of fluoresceinated goat anti-mouse IgG, cells were washed in cold PBS/BSA/azide. Controls for background fluorescence were set up similarly, but using non-reactive mouse monoclonal IgG of the corresponding class (Coulter). The percentage of specific staining in cells and the fluorescence intensities were evaluated using a Profile cell analyser (Coulter). A minimum of 10^5 cells were gated to include the macrophage subpopulation.

Binding of PE-labelled murine IgG1 molecules

PBMC (1×10^6) were incubated with 0·1–10 μg of PE-labelled mouse IgG1. Cells were washed twice with PBS/BSA/sodium azide to remove unbound antibody and analysed by flow cytometry. Scatter gates were set on the macrophage fraction. The specificity of the murine IgG1 binding was confirmed by blocking experiments using unlabelled murine IgG1 before the labelled ligand.

IL-2 assay

CTLL-2, a murine IL-2-dependent T cell line, was kept in culture medium containing 2-mercaptoethanol (5×10^{-5} M) and fed at 2-day intervals with supernatants derived from a Jurkat cell line. After several washes, 1×10^4 CTLL-2 cells were cultured in flat-bottomed microculture plates (Corning, New York, NY) and added with serial dilutions of a standard prepared with rIL-2 or samples. After 18 h, 1 μCi ^{3}H-thymidine was added. Eight hours later cells were harvested in a PhD cell harvester (Cambridge Technology, Boston, MA) and radioactivity counted in a Rackbeta counter (LKB Wallac, Sinikalliontie, Finland).

Proliferative responses of lymphocytes

PBMC (1×10^5) in complete medium were cultured in triplicate in round-bottomed microculture plates, and added with an optimal concentration (50 ng/ml) of Leu-4 MoAb. This concentration was previously determined in a group of healthy donors using a wider range of concentrations (unpublished results). In some experiments proliferative responses were also tested at several Leu-4 MoAb concentrations (see Table 2). Cells were incubated in a humidified atmosphere at 37°C, and pulsed with 1 μCi ^{3}H-thymidine 12 h before terminating the culture. Harvesting and counting was done as above. Similar dose response curves were obtained in patients and controls. Optimal proliferative responses were seen at 72 h. To measure IL-2 production, PBMC were stimulated with Leu-4 MoAb in the presence of anti-Tac MoAb (dilution 1:10 000). This antibody blocks IL-2 uptake by IL-2R, allowing more accurate measurements of IL-2 production [9].

Statistical analysis

Statistical analysis was performed by the two-tailed Student's *t*-test for unpaired samples when comparing continuous variables, and the χ^2 analysis with Yates' correction for comparing the proportion of Leu-4 responders in the study groups. $P < 0.05$ in the two-tailed test was considered statistically significant.

RESULTS

Increased number of Leu-4 responders in patients with SLE

Leu-4 responders were defined as those showing equal or higher than two-fold enhancement over baseline proliferation measured in parallel unstimulated cultures, in the presence of two different concentrations of Leu-4 MoAb. A higher number of Leu-4 responders (82%) was seen among SLE patients compared with non-SLE patients and healthy controls (Table 1). The proportion of responders among our non-SLE patients and normal controls (62%) was comparable to previous reports [10]. The responder status was consistent in time as tested repeatedly in patients and controls at a minimum of 6 month intervals. There was no correlation between the Leu-4 response status and the degree of clinical activity; four of the five inactive patients were Leu-4 responders, and in those active patients in whom clinical activity subsided there was no change in the Leu-4 response pattern (data not shown). χ^2 analysis of two by two tables correlating Leu-4 response status and presence or absence of clinical activity showed a lack of association between these two parameters ($\chi^2 = 0{\cdot}168$, $P = 0{\cdot}68$). There was no correlation between the Leu-4 response status and number of affected organs or treatment modality. All individuals were responders to OKT3 stimulation (not shown). Leu-4 non-responders were more likely to be female both in healthy donors (16/22, 72%) and SLE patients (8/8, 100%).

There was a trend for increased proliferative responses in SLE patients compared with healthy donors that was due to the high proliferative rates by individual patients (Table 2). However, at all concentrations of Leu-4 MoAb tested, differences were not statistically significant. Non-SLE patients showed diminished proliferative responses compared with SLE patients, but differences were only significant at 10 ng concentration of antibody. There was no difference in the proportions of macrophages and $CD3^+$ cells in PBMC from patients and controls; only the proportion of $CD4^+$ cells was slightly decreased compared with controls (data not shown). Among Leu-4 responders, IL-2 production was higher in SLE patients compared with healthy donors, but differences were not statistically significant: SLE = 81 ± 22 U/ml *versus* healthy controls = 56 ± 14 U/ml, mean ± s.e.m. (NS). Leu-4 non-responders showed no production of IL-2 after stimulation with this antibody, and there was a strict correlation between the Leu-4 responder status and Leu-4-induced IL-2 production (Table 3). As shown in Table 3, all Leu-4 non-responders tested showed normal OKT3-induced IL-2 production.

Table 1. Proportion of Leu-4 responders among SLE patients, non-SLE patients and healthy controls*

	Leu-4 responder				
	Yes	No		Yes	No
SLE ($n=45$)	37 (82)	8 (17)	SLE ($n=45$)	37 (82)	8 (17)
C ($n=58$)	36 (62)	22 (37)	Non-SLE ($n=37$)	23 (62)	14 (37)
($\chi^2=4{\cdot}057$, $P=0{\cdot}04$)			($\chi^2=5{\cdot}89$, $P=0{\cdot}01$)		

* Peripheral blood mononuclear cells (PBMC; 1×10^5) were cultured in triplicate in round-bottomed microculture plates and stimulated with Leu-4 MoAb. ^{3}H-thymidine incorporation was measured after 72 h. Responders were defined as those showing increases in proliferative responses at least two-fold over baseline ct/min in unstimulated cultures. Values in parentheses express the percentage of Leu-4 responders and non-responders. Statistical analysis by χ^2 with Yates' correction. P values calculated for two-tailed test.

Macrophages from SLE patients have normal helper effect for Leu-4-mediated responses

We next tested the helper effect for Leu-4 responses by macrophages from patients or controls known to be Leu-4 responders. These experiments provide indirect evidence of the degree of Leu-4 MoAb binding to FcγRII and subsequent antibody induction of CD3 cross-linking on T lymphocytes. Monocytes from either group were co-cultured with purified T cells from an unrelated healthy third-party donor. Cultures were stimulated with Leu-4 MoAb and proliferative responses measured at 72 h. Macrophages from SLE patients showed normal helper effect for Leu-4 responses compared with healthy controls (Fig. 1).

Macrophages from Leu-4 non-responders are unable to support Leu-4-induced proliferative responses by T lymphocytes from Leu-4 responders

In order to confirm the role of FcγR on accessory cells in determining the response of anti-CD3 MoAbs of the IgG1 class, we performed co-culture experiments with macrophages and T cells from a Leu-4 responder and a Leu-4 non-responder. As shown in Table 4, macrophages from a Leu-4 responder were

Table 2. Comparison of the proliferative responses by peripheral blood mononuclear cells (PBMC) from Leu-4 responders*

	Leu-4 MoAb (ng)			
	0	1	10	50
SLE ($n=37$)	1131 ± 145	2128 ± 433	8776 ± 1450	13 378 ± 2548
Non-SLE ($n=23$)	699 ± 186	1279 ± 278	4279 ± 118†	7675 ± 2245
Controls ($n=36$)	1201 ± 231	2549 ± 620	7106 ± 1006	10 604 ± 1470

* Cultures were set up as described in the legend of Table 1. Values represent mean ± s.e.m. ct/min of proliferative responses in Leu-4 responders from each group. Statistical analysis by Student's t-test for unpaired samples.

† $P = 0{\cdot}031$ (compared with SLE patients).

able to support Leu-4-induced proliferation by T cells from a non-responder. Conversely, T lymphocytes from a Leu-4 responder did not proliferate in a dose-response fashion upon stimulation with Leu-4 MoAb when co-cultured with increasing numbers of macrophages from a Leu-4 non-responder. The moderate proliferative response seen in the latter combination can be explained by allogeneic influences, since it was observed in the absence of Leu-4 MoAb (see seventh row) and did not show a dose-response pattern with increasing numbers of macrophages.

Table 3. Concordance of Leu-4 responsiveness and Leu-4-induced IL-2 production*

Experiment	Leu-4 responder	Leu-4 non-responder	IL-2 (U/ml)
1		+	0·5 (218)
2		+	1·0 (229)
3		+	0·5 (180)
4		+	1·0 (189)
5		+	8 (632)
6	+		64 (226)
7	+		191 (238)
8	+		82 (79)
9	+		75 (105)
10	+		58 (183)
11	+		133 (158)
12	+		59 (230)
13	+		110 (190)

* Leu-4 responsiveness was determined in 72-h cultures as detailed in Patients and Methods. The production of IL-2 was measured at 48 h in parallel cultures activated with Leu-4 MoAb (50 ng) or OKT3 MoAb (10 ng). Levels of IL-2 induced by OKT3 MoAb are shown in parentheses.

Comparison of Fc receptor expression in monocytes from SLE patients and controls

The proportion of macrophages present in mononuclear cells was comparable in SLE patients and healthy controls, whereas non-SLE patients showed lower numbers of macrophages compared with healthy donors (data not shown). The number of macrophages bearing FcγRI was increased in SLE patients compared with healthy controls (Table 5), but the proportion of macrophages bearing FcγRII and FcγRIII was similar in all three groups. The intensity of anti-FcR MoAb staining, as measured by the log of mean channel fluorescence, was similar

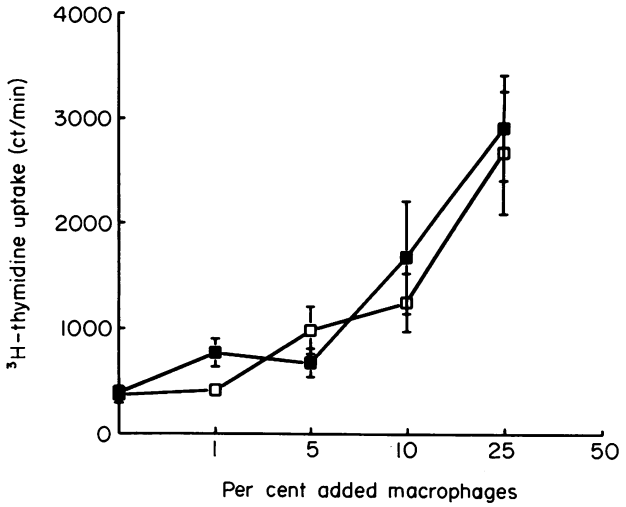

Fig. 1. T cells from either of two healthy donors were co-cultured with increasing numbers of macrophages derived from 14 different SLE patients or unrelated healthy controls. Macrophages were pretreated with mitomycin-C as described in Patients and Methods. Cultures were stimulated with Leu-4 MoAb (50 ng) and proliferative responses measured at 72 h. □, Controls ($n = 14$); ■, SLE patients ($n = 14$).

Table 4. Response to Leu-4 MoAb in co-cultures of T lymphocytes with macrophages from Leu-4 responders and non-responders*

Medium	Leu-4 MoAb	Autologous macrophages (%)	Allogeneic macrophages (%)	Leu-4 non-responder	Leu-4 responder
+	−	−	−	1472	1275
+	+	−	−	1737	1321
+	−	25	−	1698	1644
+	+	5	−	1724	1150
+	+	10	−	889	3952
+	+	25	−	835	9129
+	−	−	25	1175	4386
+	+	−	5	5943	4612
+	+	−	10	10547	3608
+	+	−	25	17839	2777

* T cells (1×10^5) and varying numbers of macrophages from a Leu-4 non-responder and a Leu-4 responder healthy donor were co-cultured in triplicate in a U-bottomed microculture plate. Cells were isolated as described in Patients and Methods. Macrophages were irradiated with 30 Gy before being added to cultures. Leu-4 MoAb (50 ng/ml) was added as indicated at the beginning of culture. As control for responses related to allogeneic influences, 25% of allogeneic macrophages were added in the absence of Leu-4 MoAb (see row seven). Cells were cultured during 72 h and ^{3}H-thymidine added 18 h before terminating the culture. Shown are mean ± s.e.m. ct/min of a representative experiment.

Table 5. FcR expression in monocytes from SLE patients and controls*

	FcγRI	FcγRII	FcγRIII
SLE patients ($n=30$)	79±3	85±2	47±4
Non-SLE patients ($n=20$)	74±2	87±2	45±6
Healthy ($n=30$)	68±3†	90±2 (NS)	44±4 (NS)

* Mononuclear cell preparations were stained with MoAb specific for each FcγR type and macrophages were gated for flow cytometric analysis. Shown are the mean±s.e.m. of percentages of positive cells.

† $P=0.031$ comparing FcγRI in healthy donors and SLE patients.

in SLE patients, non-SLE controls and healthy donors, except for relatively lower values for FcγRII in the SLE group (2·6±0·20 in SLE *versus* 4·1+0·37 in healthy donors, $P=0.0005$).

We also examined the binding of murine IgG1 molecules by macrophages from Leu-4 responders of the SLE and control groups. To this end, we incubated PBMC with PE-labelled murine IgG1 (10 μg/ml) and tested the direct fluorescence by flow cytometry. Mean channel fluorescence readings were comparable in both groups: 52·8±6·5 in SLE *versus* 58·7±4·9 in healthy controls, NS ($n=8$). The specificity of ligand binding was confirmed by blocking experiments in which cells were preincubated with unlabelled murine IgG1 (data not shown).

DISCUSSION

In this study we show that Venezuelan SLE patients are more likely to respond to Leu-4, an anti-CD3 MoAb of the IgG1 class, than normal controls. Our findings can not be explained by enhanced sensitivity of SLE T cells to stimulation with Leu-4 MoAb, since Leu-4-induced proliferation by Leu-4 responders in most SLE patients was comparable to controls. Further, co-culture experiments showed a similar helper effect by SLE and control macrophages on Leu-4-activated T cells from a healthy third-party donor. Also, the binding of PE-labelled murine IgG1 by macrophages of Leu-4 responders was similar in patients and controls. Finally, the proportion of macrophages bearing FcγRII did not differ in the three groups. These findings seem to suggest that our patients are genetically more susceptible than their healthy counterparts to express the phenotypic variant of FcγRII, which is capable of binding this class of murine immunoglobulin molecules.

Our findings were not related to the degree of clinical activity, and can not be explained as a non-specific effect related to abnormal immune response or corticosteroid treatment, since a group of patients with diverse immune-related conditions receiving similar doses of corticosteroids showed a Leu-4 response pattern comparable to healthy donors.

Approximately 70% of healthy individuals are able to respond to anti-CD3 MoAb of the IgG1 class [1,10,11]. The ability to respond depends on the presence of FcγR with affinity for murine IgG1 molecules and the capability of inducing cross-linking of the CD3 complex on T cells [4]. In the presence of macrophages from Leu-4 responders, T cells from non-responders are also able to proliferate to the same extent as T cells from responders [4,10,11] (Table 4). The proportion of macrophages bearing FcγRII was similar in SLE patients and controls (Table 4). It has previously been demonstrated that the density of FcRII and the relative proportion of macrophages bearing it is similar in healthy Leu-4 responders and non-responders [12]; the Leu-4 responder status depends on the presence of a variant FcRII molecule with an altered isoelectric focusing mobility [12] due to a discrete amino acid substitution in its extracellular domain, and is independent of the total number of FcγRII molecules present on macrophages [13].

A previous study by van Wauwe & Goosens showed that healthy female donors were more likely to be Leu-4 non-responders [10]. In our study 72% of the 22 healthy and 100% of the eight SLE Leu-4 non-responders were female, confirming a potential gender influence on Leu-4 responsiveness among healthy donors. However, if this factor had any influence on our results we would expect a higher number of Leu-4 non-responders in the predominantly female SLE group, suggesting that the presence of this disorder may abolish the gender effect.

In the present study we show that contrary to OKT3-mediated responses [5], Leu-4-induced proliferation and IL-2 production by Leu-4 responders were comparable in SLE patients and controls. These results suggest that anti-CD4 MoAbs of the IgG2 class may permit a more efficient CD3 cross-linking than IgG1 anti-CD3 antibodies, or induce a different set of second signals from FcR-bearing accessory cells in SLE patients. Alternatively, the enhanced FcγRI expression on SLE macrophages (Table 5) may facilitate responses by anti-CD3 MoAbs of the IgG2a subclass, the one preferentially bound by this type of receptor.

Our results contrast with those by Kaneoka *et al.* [14] showing decreased Leu-4-mediated responsiveness in SLE patients. It is possible that patient selection may have affected the results in both studies. Our cases were mostly in-patients with active disease, suggesting a more aggressive pattern. Eighteen of our patients had biopsy-proven renal disease, six central nervous system manifestations and seven polyserositis. Also, we isolated T cells by negative selection, whereas they used E-rosetting, a method based on the binding of sheep erythrocyte to the CD2 membrane receptor. Perturbation of these receptors may send negative signals [15] that may counterbalance CD3-mediated responses by SLE T cells. Finally, the ethnic composition of our patient group, Hispanic *versus* Asian in Kaneoka's report, may have contributed to the discrepant results. It is known that responses in IgG1 class anti-CD3 MoAbs vary in different ethnic populations, with lower frequencies observed in individuals of Asian origin [16]. As far as we know, no previous data exist on Leu-4 responses by healthy individuals of Hispanic origin, but our results suggest a lower proportion of Leu-4 responders compared with Caucasian donors, closer to that previously reported in Asian donors.

Recent studies have demonstrated heterogeneity of FcγR in human macrophages with regard to affinity for murine IgG molecules [17]. FcRII (CD32) is a 40-kD glycoprotein with multiple isoforms and allotypes. There are two functionally polymorphic variants, one designated $FcRII^{HR}$ that can bind mouse IgG1 and corresponds to the subtype conferring Leu-4 responsiveness. The second variant, designated $Fc\gamma RII^{LR}$, is unable to bind murine IgG1 molecules and determines the Leu-4 non-responder status. It has recently been suggested that this FcγR polymorphism is probably relevant to the regulation of human immunoglobulin subclasses. Warmerdam *et al.* [18]

showed that FcγRII^{LR} but not FcγRII^{HR} can bind human IgG2a efficiently. This may explain why healthy donors bearing FcγRII of the Leu-4 non-responder phenotype had lower levels of IgG2a [19]. These data suggest that FcγR may play a fundamental role in immunoglobulin turnover and isotypic regulation. Abnormalities in the handling of immunoglobulin subclass molecules by FcγR may be relevant to the pathogenesis of SLE by means of various mechanisms such as up-regulation of pathogenic autoantibodies, triggering of antibody-dependent cytotoxicity mechanisms by FcγR-bearing cells, or by alteration in the clearance of circulating immune complexes.

In favour of this notion, SLE patients seem to have a more promiscuous profile of isotypic responses to common antigens [20]. Further, anti-Sm antibodies, a specific marker for SLE, are predominantly of the IgG2a subclass [21]. Also, two previous studies showed enhanced binding of human IgG by FcγR from SLE patients [22,23]. Recent reports showing that FcγR are themselves targets of autoantibodies in SLE [24] and that binding through FcγR can trigger subcellular signals critical for macrophage activation [25] provide further interest in this area. Our study gives rationale to further work oriented to understanding the handling of IgG subclasses by FcγR.

A recent work showed comparable restriction fragment length polymorphisms in genes coding FcγRII in Caucasian SLE patients and controls [26]. It is conceivable that Venezuelan SLE patients may have allelic variants of genes coding components of FcγRII predisposing to a Leu-4 responder phenotype. Whether this qualitative difference relates to known abnormalities in SLE macrophage cell function awaits further studies.

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