Autoimmunity, April 2009; 42(4): 263–265

ISSN 0891-6934 print/1607-842X online
DOI: 10.1080/08916930902827942

informa
healthcare

BRIEF DEFINITE REPORT

Total and biologically active CD154 in patients with SLE

JUAN B. DE SANCTIS[1], JENNY V. GARMENDIA[1], RICARDO CHAURIO[1,2], MERCEDES ZABALETA[1], & LILIANA RIVAS[1]

[1]*Facultad de Medicina, Instituto de Inmunología, University of Central de Venezuela, Caracas, Venezuela, and* [2]*Department of Internal Medicine 3, Institute for Clinical Immunology, University of Erlangen-Nuremberg, D-91054 Erlangen, Germany*

(*Submitted 6 October 2008; revised 5 January 2009; accepted 7 January 2009*)

Abstract
CD154, a member of the tumor necrosis factor receptor family, is involved in several biological responses. In the sera of systemic lupus erythematosus (SLE) patients, the levels of sCD154 have been shown to be increased, however, few reports have dealt with the biologically active tetramer. Here, we assessed the biological activity of the serum CD154 tetramer using bioassays for BC activation and production nitrite or peroxide. The patients showed a markedly increased total sCD154 serum concentration (12.5 ± 8.2 vs. 3.9 ± 1.2 ng/ml; $p < 0.001$). ba-sCD154 was significantly increased in non-treated patients (7.4 ± 3.4 ng/ml, $n = 22$; $p < 0.001$) and patients with the highest SLE disease activity index (SLEDAI) scores (5.3 ± 2.9 ng/ml, $n = 8$), but not in stable patients (1.3 ± 1.2 ng/ml, $n = 30$) whose values were similar to normal healthy donors (NHD; 0.8 ± 0.2 ng/ml). Patients with SLEDAI above 8 that recovered after successful treatment displayed significantly decreased levels of ba-sCD154. We conclude that the bioassay is a useful tool discriminating active and stable SLE, as well as non-treated patients.

Keywords: *ba-sCD154, SLE, ELISA*

Introduction

Several hypotheses have been proposed to explain the genesis of autoimmune disorders. Hyperactive non-apo memory cells against specific or non-specific autoantigen have been a common characteristic of autoimmune disorders [1]. Certain antigens play important roles in understanding autoimmunity. Among them, the CD40–CD154 interaction has been involved in different key events in innate and acquired immune response [2]. In most autoimmune disorders like systemic lupus erythematosus (SLE), CD154 is over expressed in CD4+ memory T cells (TC). In addition, ba-sCD154 has also been encountered [2–8]. CD154 blockage with specific antibodies (Ab) has been shown to be a successful experimental treatment for several diseases from atherosclerosis to SLE [6].

Only few reports, like that of Vakkalanka et al. [4], have addressed the issue of the biological activity of sCD154 and quantified the tetramer. They suggested that this molecule is crucial for the biological response in SLE. Garmendia et al. [9] have performed bioassays to determine the amount of ba-sCD154 as compared to total sCD154 detectable by enzyme-linked immunosorbent assay (ELISA). In patients with chronic idiopathic urticaria, ba-sCD154 discriminated patients with positive and negative autologous serum skin test and thus provided evidence for immune cell activation in this chronic disease.

Materials and methods

Blood samples were obtained from 60 patients with SLE and 60 controls (see manuscript of Liliana Rivas this issue). A written consent was obtained from all the individuals involved in the study and the study was approved by the local Bioethical Committee. Some characteristics of the population are depicted on Table I. Serum sCD154 levels were assessed using a commercial sandwich ELISA assay (Chemicon

Correspondence: Dr J. V. Garmendia. Facultad de Medicina, Instituto de Inmunología, University of Central de Venezuela, Apartado 50209, Sabana Grande, Caracas 1050-A, Venezuela. Tel: 58 212 6934767. Fax: 58 212 6932815. E-mail: jenny.garmendia@gmail.com

Table I. Patients' characteristics and total soluble CD154.

		SLE	
	NHD ($n = 100$)	Non-treated ($n = 22$)	Treated ($n = 38$)
Age	33 ± 12	34 ± 12	31 ± 5
Female	85 (85%)	20 (91%)	35 (92%)
SLEDAI	–	9.5 ± 4.6	7.1 ± 3.2
sCD154 (ng/ml)	3.9 ± 1.2	11.9 ± 3.6⋆	6.7 ± 2.3⋆⋆
ba-sCD154 (ng/ml)	0.8 ± 0.2	7.4 ± 3.4⋆	2.9 ± 1.5⋆⋆

Note: ⋆$p < 0.001$ as compared to NHD; ⋆⋆$p < 0.01$ as compared with NHD, assessed by ANOVA and Bonferroni post test.

International, Temecula, CA, USA). The ELISA did not distinguish ba-sCD154 and non-active forms of sCD154.

The biological activity was performed as described previously [9]. Briefly, we assessed proliferation of purified BC, and nitrite and peroxide production of RAW 264.7 cells and Nph, respectively [10]. The specificity of the biological activity was confirmed by addition of Ab or Ig-CD40 (Ancell, Bayport, MN, USA), both specifically neutralizing the activity of sCD154. Surface expression of CD154 (Ancell) was assessed by flow cytometry.

Results and discussion

Table I represents the characteristics of the patients and the values of total and ba-sCD154. The untreated patients displayed significantly higher values of ba-sCD154 than NHD (7.4 ± 3.4 ng/ml vs. NHD 0.8 ± 0.2 ng/ml, $n = 22$; $p < 0.001$. These patients were grouped according to SLE disease activity index (SLEDAI) index with a threshold of 8. Those with SLEDAI > 8 displayed a significant higher ba-sCD154 level than those SLEDAI < 8 (14.3 ± 3.6 ng/ml, $n = 10$ and 5.5 ± 2.8 ng/ml, $n = 12$; $p < 0.01$). For treated patients with SLEDAI > 8 ($n = 8$) and SLEDAI < 8 ($n = 30$) ba-sCD154 values were 5.3 ± 2.9 and 1.3 ± 1.2 ng/ml, respectively ($p < 0.01$).

The patients with the highest SLEDAI indices above 8 ($n = 18$), either treated or non-treated, were longitudinally studied for 6 months (6 times) after treatment was started or changed. Their ba-sCD154 levels dramatically decreased from 9.8 ± 3.5 to 2.7 ± 2.1 ng/ml ($p < 0.01$). The decrease was gradual, but constant and paralleled the decrease in autoantibodies and the increase in C3 and C4, although these changes were not statistically significant ($r = 0.15$ and 0.2). These changes in sCD154 may reflect immune cell deactivation upon successful treatment [2–6].

Several questions remain unanswered, e.g. concerning the fate of ba-sCD154, since it has been suggested that it may be degraded or modified. We have studied a group of patients ($n = 35$), in which

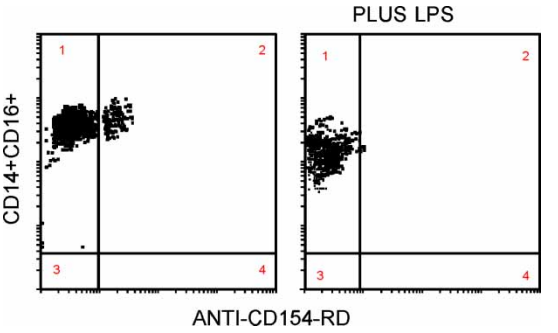

Figure 1. Effect of LPS on CD14+ CD16+ cells. Note: The figure represents a flow cytometry assessment of a SLE patient with high CD154 expression in the CD14CD16 population treated with LPS. A marked decrease in CD154 is observed.

leukocyte surface CD154 was assessed by flow cytometry. Interestingly, patients, but not controls, had the highest expression of CD154 in CD14+ CD16+ MΦ and not in CD4+ TC (4.5 ± 1.5% vs. 0.2 ± 0.3%; $p < 0.05$). Cells were stimulated with 1 μg/ml lipopolysaccharide (LPS) for 5′ and subsequently assayed for CD154 expression. Most of the CD154 was liberated from the membrane upon LPS treatment, since the molecule was captured by fluorescent beads covered with anti-CD154. As shown in Figure 1. These results are in agreement with those of Katsiari et al. [11] and, as postulated by Schiffer et al. [12], this parameter may be useful identifying lupus nephritis.

It can be concluded that ba-sCD154 is an important molecule for cell activation. It may be used to assess systemic immune activation and seems to be predominantly secreted by CD14+ CD16+ inflammatory MΦ which, upon deactivation or tissue redistribution, express CD40.

Acknowledgements

This work was supported by a grant from CONICIT/FONACIT grants S12000000149 and G2005000389.

References

[1] Kotzin BL. Mechanisms of autoimmunity. In: Rich RR, Fleisher TA, Shearer WT, Kotzin BL, Schroeder Jr, HW, editors. Clinical immunology principles and practice. Chapter 58. 2nd ed. St Louis: Mosby Publishers; 2001. p 58.1–58.13.

[2] Toubi E, Shoenfeld Y. The role of CD40–CD154 interactions in autoimmunity and the benefit of disrupting this pathway. Autoimmunity 2004;37(6–7):457–464.

[3] Carneiro-Sampaio M, Liphaus BL, Jesus AA, Silva CA, Oliveira JB, Kiss MH. Understanding SLE physiopathology in the light of primary immunodeficiencies. J Clin Immunol 2008;28(Suppl 1):S34–S41.

[4] Vakkalanka RK, Woo C, Kirou KA, Koshy M, Berger D, Crow MK. Elevated levels and functional capacity of sCD40L in SLE sera. Arthritis Rheum 1999;42(5):871–881.

[5] Kato K, Santana-Sahagún E, Rassenti LZ, Weisman MH, Tamura N, Kobayashi S, Hashimoto H, Kipps TJ. The sCD154 in SLE. J Clin Invest 1999;104(7):947–955.
[6] Ferro D, Pignatelli P, Loffredo L, Conti F, Valesini G, D'Angelo A, Violi F. sCD40L plasma levels in patients with SLE: Modulation by APL. Arthritis Rheum 2004;50(5): 1693–1694.
[7] Goules A, Tzioufas AG, Manousakis MN, Kirou KA, Crow MK, Routsias JG. Elevated levels of sCD40L in serum of patients with systemic autoimmune diseases. J Autoimmun 2006;26(3):165–171.
[8] Komura K, Fujimoto M, Matsushita T, Yanaba K, Kodera M, Kawasuji A, Hasegawa M, Takehara K, Sato S. Increased serum sCD40L levels in patients with systemic sclerosis. J Rheumatol 2007;34(2):353–358.
[9] Garmendia JV, Zabaleta M, Blanca I, Bianco NE, De Sanctis JB. Total and biologically active serum-sCD40L in patients with chronic idiopathic urticaria. Allergy Asthma Proc 2004; 25(2):121–125.
[10] Lara L, Rivera H, Pérez-P C, Blanca I, Bianco NE, De Sanctis JB. Low density lipoprotein receptor expression and function in human polymorphonuclear leukocytes. Clin Exp Immunol 1997;107:205–212.
[11] Katsiari CG, Liossis SN, Dimopoulos AM, Charalambopoulo DV, Mavrikakis M, Sfikakis PP. CD40L overexpression on TC and Mo from patients with SLE is resistant to calcineurin inhibition. Lupus 2002;11(6):370–378.
[12] Schiffer L, Bethunaickan R, Ramanujam M, Huang W, Schiffer M, Tao H, Madaio MP, Bottinger EP, Davidson A. Activated renal macrophages are markers of disease onset and disease remission in lupus nephritis. J Immunol 2008;180(3): 1938–1947.