ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/6736192

Herpesvirus saimiri (HVS)-transformed T-cell lines: A method to study mucosal T cells in inflammatory bowel disease

Article *in* Scandinavian Journal of Gastroenterology · December 2006

DOI: 10.1080/00365520600670083 · Source: PubMed

CITATIONS
2

READS
27

11 authors, including:

Noemí Aguilera-Montilla
Spanish National Research Council
25 PUBLICATIONS **169** CITATIONS

SEE PROFILE

Mercedes Lopez-Santalla
Centro Investigaciones Energéticas, Medioambientales y Tecnológicas
38 PUBLICATIONS **174** CITATIONS

SEE PROFILE

Angeles Mencía
University Carlos III de Madrid
32 PUBLICATIONS **710** CITATIONS

SEE PROFILE

José M. Martin-Villa
Complutense University of Madrid
123 PUBLICATIONS **1,610** CITATIONS

SEE PROFILE

Some of the authors of this publication are also working on these related projects:

MHC evolution View project

DRB6 gene discovery View project

All content following this page was uploaded by Mercedes Lopez-Santalla on 24 November 2015.

The user has requested enhancement of the downloaded file.

Scandinavian Journal of Gastroenterology, 2006; 41: 1361–1363

Taylor & Francis
Taylor & Francis Group

SHORT REPORT

Herpesvirus saimiri (HVS)-transformed T-cell lines: A method to study mucosal T cells in inflammatory bowel disease

NOEMÍ AGUILERA-MONTILLA[1], MERCEDES PÉREZ-BLAS[1], ANNA PAOLA VALERI[1], MERCEDES LÓPEZ-SANTALLA[1], CRISTINA RODRÍGUEZ-JUAN[1], ÁNGELES MENCÍA[1], GREGORIO CASTELLANO[2], MARÍA LUISA MANZANO[2], BEGOÑA CASIS[2], FERNANDO SÁNCHEZ[2] & JOSÉ MANUEL MARTÍN-VILLA[1]

[1]*Inmunología, Facultad de Medicina, Universidad Complutense de Madrid, Spain, and* [2]*Servicio de Gastroenterología, Hospital Universitario 12 de Octubre, Madrid, Spain*

Introduction

Alterations in the mechanisms regulating the immune response are key elements in inflammatory bowel disease (IBD, comprising Crohn's disease (CD) and ulcerative colitis (UC)). Although the precise aetiology remains unknown, T lymphocytes play a crucial role in its pathogenesis. Initially, descriptions of the immunological features in IBD focused on peripheral blood lymphocytes. Since these data do not necessarily reflect the situation at the mucosal milieu, it is important to assess the phenotypic profile and activation state of immune cells at this level.

We have used a common lymphotropic virus of squirrel monkeys, *Herpesvirus saimiri* (HVS), to immortalize T cells of intestinal mucosal origin from patients and healthy individuals as controls. HVS-transformed T lymphocytes become antigen- and mitogen-independent for their continuous growth and upon stimulation with membrane or transmembrane stimuli, these cells show normal downstream functional responses [1,2].

Methods

Intestinal tissue explants (colonic samples) were obtained by colonoscopy from patients with IBD. Control specimens consisted of normal sigmoid colon samples obtained from individuals undergoing endoscopy for colonic motility disorders (constipation, diarrhoea, irritable bowel syndrome) or bleeding haemorrhoids. Mucosa was rinsed in 0.09% sodium chloride containing 2% antibiotic. Mucus was removed by treatment with RPMI 1640, and 1mM dithiothreitol, for 30 min. The pieces were then resuspended in RPMI 1640 with 10% foetal calf serum (FCS) and 1% L-glutamine, supplemented with 50 U/ml recombinant human IL-2 (rhIL-2, TECIN, kindly provided by Hoffmann-La Roche, Basel, Switzerland) and seeded into 6-well microplates. Lymphocytes were then collected from the supernatant, washed and used for the transformation procedure, as previously described [2]. One CD-derived T-cell line, four UC lines and two healthy lines were finally obtained.

HVS-derived cell lines were stimulated with membrane or trans-membrane stimuli. Thus, monoclonal antibodies to CD3, (α-CD3), α-CD2 or α-CD28, alone or in combination with other mitogenic substances such as interleukin-2 (IL-2), phorbol 12-myristate 13-acetate (PMA) or ionomycin, were used.

A total of 90,000 cells per well were seeded in triplicate in a 96-well plate (Sarstedt), stimulated for 48 h, then pulsed with 1 μCi ^{3}H-thymidine (Moravek Biochemicals, Brea, Calif., USA), left for a further 18 h and harvested to evaluate incorporation in cellular DNA in a beta counter (Matrix 96; Packard, Canberra Company, Canberra, Australia). Results are expressed as mean counts per minute (cpm) [1,2].

Correspondence: José M. Martin-Villa, Inmunología, Facultad de Medicina, Pabellón V, Planta 4ª, Universidad Complutense de Madrid, ES-28040 Madrid, Spain. Tel: +34 91 3941 642. Fax: +34 91 3941 641. E-mail: autoinmunidad@med.ucm.es

(Received 13 December 2005; accepted 2 March 2006)

ISSN 0036-5521 print/ISSN 1502-7708 online © 2006 Taylor & Francis
DOI: 10.1080/00365520600670083

The experiments were carried out with the approval of the ethics committee of the institution.

Results

CD3-specific monoclonal antibodies (α-CD3) yielded higher counts in CD and UC patients than in control individuals ($189 \pm 25 \times 10^3$ cpm in CD and $164 \pm 22 \times 10^3$ cpm in UC versus $98 \pm 11 \times 10^3$ cpm in controls, $p < 0.01$, see Figure). If α-CD3+IL-2 or α-CD3+α-CD2 stimuli were considered, significance was reached when compared with control cells for UC in the former combination ($150 \pm 23 \times 10^3$ cpm versus $102 \pm 11 \times 10^3$ cpm, $p < 0.05$) and for CD in the latter ($224 \pm 43 \times 10^3$ cpm versus $129 \pm 16 \times 10^3$ cpm, $p < 0.05$).

When dissecting the CD2-based pathway, α-CD2+PMA revealed a significant proliferation in CD lines compared to healthy subjects ($117 \pm 10 \times 10^3$ cpm versus $50 \pm 9 \times 10^3$ cpm, $p < 0.01$), whereas significance was reached for UC when α-CD2+α-CD28 was used ($121 \pm 43 \times 10^3$ cpm versus $54 \pm 9 \times 10^3$ cpm, $p < 0.05$).

Use of ionomycin consistently resulted in higher counts in patients as compared to control lines, whether alone ($28 \pm 4 \times 10^3$ cpm in CD, $17 \pm 4 \times 10^3$ cpm in UC and $9 \pm 2 \times 10^3$ cpm in control cells, $p < 0.01$ and $p < 0.05$, respectively) or in combination with PMA ($66 \pm 18 \times 10^3$ cpm in CD, $58 \pm 13 \times 10^3$ cpm in UC and $29 \pm 5 \times 10^3$ cpm in control cells, $p < 0.05$).

Comment

We report on the availability of T-cell lines of mucosal origin from IBD patients by means of HVS transformation. These lines allow us to carry out experiments that would otherwise be impossible to carry out given the scarcity of T lymphocytes obtained from tissue explants.

Mucosal lines from patients proliferate more readily than from healthy controls, irrespective of whether membrane (α-CD3, α-CD2), or transmembrane (PMA, ionomycin) stimuli were used (see Figure). Since these lines are grown *ex vivo* for several months, isolated from their natural intestinal milieu, T cells can proliferate in the absence of interfering factors (secreted by other cells, such as intestinal epithelial cells [3], or immunosuppressive drugs used in these patients). The molecular bases underlying this inherent active response are presently unknown, but the cell lines shown herein may be adequate tools to dissect it.

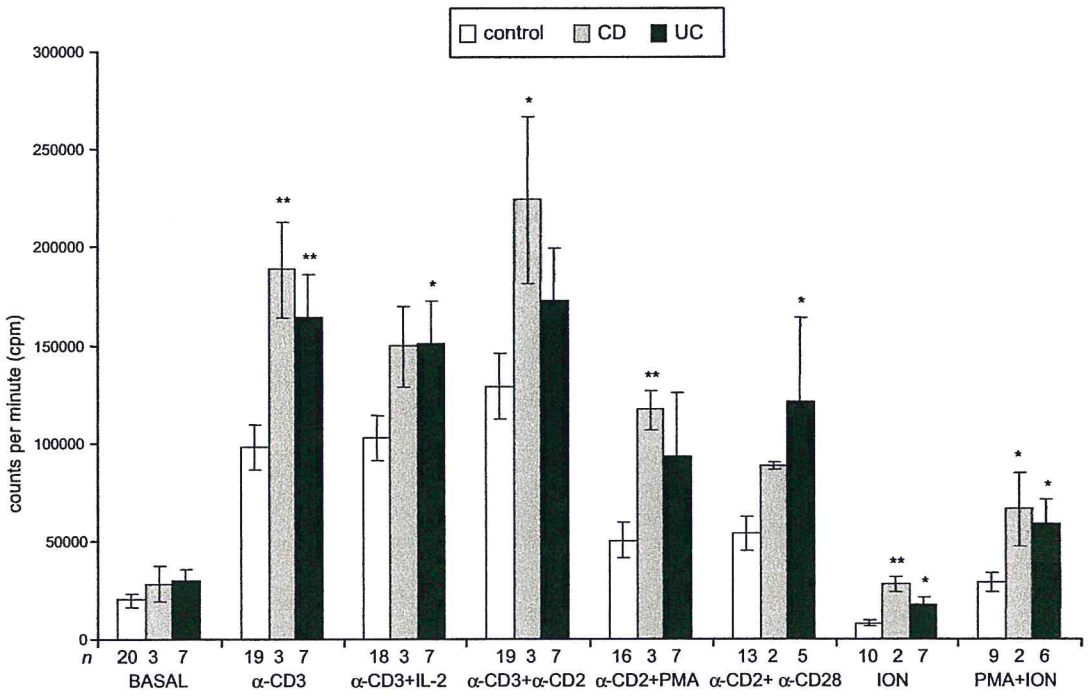

Figure 1. Proliferative response in counts per minute (cpm) of *Herpesvirus saimiri* (HVS)-transformed T-cell lines of mucosal origin. One Crohn's disease (CD)-derived T-cell line, four ulcerative colitis (UC) lines and two control lines were analysed. Results obtained are expressed as mean values ± standard error of the mean (SEM). The two-tailed Mann-Whitney, two-sample test was used to compare results between cancer and control HVS lines. Significance was reached when a p-value of less than 0.05 was obtained; n represents the number of experiments carried out with each stimulus; PMA = phorbol 12-myristate 13-acetate; $^*p < 0.05$; $^{**}p < 0.01$.

The major working hypothesis concerning the pathogenesis of IBD is that the disease is caused by an abnormal and uncontrolled mucosal immune response to one or more normally occurring gut constituents. Our results suggest that mucosal T cells from patients present an inherent over-responsive state, which may be an essential feature of IBD pathogenesis.

Acknowledgements

This work was supported in part by a grant from the Fondo de Investigaciones Sanitarias (99/0999) and a Comunidad Autónoma de Madrid grant (083/0009/1998). NA-M, APV, ML-S and CR-J are recipients of grants from Ministerio de Educación, Cultura y Deporte (FPU AP 99/212668696 (NA-M)), Fondo de Investigaciones Sanitarias (N° 99/0999 (APV) and Comunidad Autónoma de Madrid (N° 01/0264/02 [ML-S]) and N° 01/0774/98 [CR-J].

N A-M and M P-B can be considered as joint first authors.

References

[1] Valeri AP, Perez-Blas M, Gutierrez A, Lopez-Santalla M, Aguilera N, Rodriguez-Juan C, et al. Intrinsic defects explain altered proliferative responses of T lymphocytes and HVS-derived T-cell lines in gastric adenocarcinoma. Cancer Immunol Immunother 2003;52:708–14.

[2] Aguilera-Montilla N, Perez-Blas M, Valeri AP, Lopez-Santalla M, Rodriguez-Juan C, Mencia A, et al. Higher proliferative capacity of T lymphocytes from patients with Crohn disease than from ulcerative colitis is disclosed by use of Herpes virus saimiri-transformed T-cell lines. Scand J Gastroenterol 2004; 39:1236–42.

[3] Christ AD, Colgan SP, Balk SP, Blumberg RS. Human intestinal epithelial cell lines produce factor(s) that inhibit CD3-mediated T-lymphocyte proliferation. Immunol Lett 1997;58:159–65.

View publication stats