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SHORT COMMUNICATION

Herpesvirus saimiri transformation may help disclose inherent functional defects of mucosal T lymphocytes in patients with gastric adenocarcinoma

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To dissect the phenotypic and functional features of mucosal T lymphocytes in patients with gastric adenocarcinoma, we have used the *Herpesvirus saimiri* transformation procedure to achieve T-cell lines from gastric origin. Once achieved, cell function was assessed by *in vitro* stimulation with mitogens. CD2-specific monoclonal antibodies (α-CD2), alone or in combination with interleukin (IL)-2, rendered fewer counts in patients (34 408 ± 3965 and 52 157 ± 6473 c.p.m., respectively) than in controls (67 471 ± 11 755 c.p.m., $P<0.01$ and 77 864 ± 12 545 c.p.m., $P<0.05$, respectively). Likewise, CD3-based responses were defective in cancer cell lines: α-CD3 (54 794 ± 9269 vs 86 104 ± 10 341 c.p.m., $P<0.01$), α-CD3+IL-2 (57 789 ± 8590 vs 88855 ± 8516 c.p.m., $P<0.01$) and α-CD3+α-CD2 (52 130 ± 7559 vs 120 852 ± 16 552 c.p.m., $P<0.01$). Finally, IL-2 failed to adequately stimulate patient cell lines (39 310 ± 4023 vs 60 945 ± 9463 c.p.m., $P<0.05$). These results suggest that mucosal T lymphocytes in cancer patients are inherently impaired in their proliferative ability. This may be crucial in the control of tumour growth.

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Several alterations at the phenotypic or functional level have been described in T lymphocytes of patients with cancer,[1–4] and they have been implicated as causes of cancer dissemination, despite the expression of tumoral antigens on neoplastic cells.

In some types of cancer, isolation and growth of T cells from tissue samples are required to analyse their role in limiting the expansion of solid tumours, but since their purification is complicated, the use of T-cell lines may be useful to dissect their functionality.

We took advantage of the transformation procedure with a common lymphotropic virus, the *Herpesvirus saimiri* (HVS),[5] to immortalize T lymphocytes of mucosal origin from patients with gastric adenocarcinoma. Previous results published by our group[6] showed that HVS-derived T-cell lines from peripheral blood samples mirrored the defects found on fresh T cells. The validity of HVS-transformed cells as experimental models has been widely discussed elsewhere.[7]

T-cell lines were obtained from gastric mucosal samples of patients with gastric adenocarcinoma and control individuals, and their proliferative response upon mitogenic stimulation was analysed.

RESULTS

T lymphocytes of gastric origin from six patients were successfully transformed with the HVS, and eight different cell lines were achieved (four patients rendered one line each, and other two patients two lines each. See Table 1 for details). Cell lines are either $CD4^+$ or $CD8^+$ and, in this instance, six are $CD4^+$ and two are $CD8^+$. The transformation of cells from control intestinal samples rendered two different cell lines, one being $CD4^+$ and the other $CD8^+$.

Herpesvirus saimiri-derived T cells from cancer patients showed defective responses upon mitogenic stimulation, as compared with control cell lines (Figure 1). Monoclonal antibodies to CD2 (α-CD2), whether alone or in combination with interleukin (IL)-2, rendered fewer counts in cancer patients (34 408 ± 3965 and 52 157 ± 6473 c.p.m., respectively) than in control subjects (67 471 ± 11 755 c.p.m., $P<0.01$ and 77 864 ± 12 545 c.p.m., $P<0.05$, respectively). Likewise, CD3-mediated stimulation also revealed poor responses in cell line of cancer patients: α-CD3 (54 794 ± 9269 vs 86 104 ± 10 341 c.p.m., $P<0.01$), α-CD3+IL-2 (57 789 ± 8590 vs 88855 ± 8516 c.p.m., $P<0.01$) and α-CD3+α-CD2 (52 130 ± 7559 vs 120 852 ± 16 552 c.p.m., $P<0.01$). Finally, IL-2 alone also stimulates to

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a lesser extent cell lines from patients (39 310 ± 4023 c.p.m.) than from control individuals (60 945 ± 9463 c.p.m., $P<0.05$).

DISCUSSION

Cell lines from patients showed diminished responses to membrane-based stimuli (Figure 1), indicating a dysfunction of the signalling pathways. All three stimuli shown share in common the recruitment of the *Lck* kinase,[8,9] a central element in the initiation steps leading to the full activation of T lymphocytes. A malfunction of this kinase would render T cells unable to respond *in vitro* to several stimuli and, *in vivo*, to foreign or tumoral antigens. In fact, a diminished *Lck* expression has been reported in animal models of cancer.[2]

Transformation with HVS activates the Lck tyrosine kinase by binding of the Tip protein of the virus, and antigen-independent growth of the cell lines is mediated by CD2 interaction with its cognate ligand, CD58, on the cell's surface.[10] Surprisingly though, T-cell lines from patients responded poorly when stimulated via CD2, and either viral-induced Lck activity is not adequate or another signalling kinase is altered in the patients. Moreover, the fact that costimulation via CD3 and CD2 shows significantly lower counts in patients suggests that either the defective CD3 or CD2 pathways are unable to render diseased cells to a normal state upon costimulation or, alternatively, that assembly of an as-yet-unidentified functional signalling complex, specific for CD2 and CD3 costimulation, fails to take place in cell lines from patients but not from healthy volunteers. Biochemical studies are required to confirm this hypothesis, and availability of the cell lines shown in this work is a useful element for future research.

The fact that these alterations are found in cell lines grown *in vitro* for several months and, therefore, in the absence of any tumour-derived factor, and that they are found in the cell lines of patients irrespective of their origin, tissue (present work) or blood,[6] strongly reinforces the suggestion that intrinsic defects, and not tumour factors, are the main cause of the limited proliferative capacity of T lymphocytes in patients with cancer. In this situation, tumoral cells would find a hampered immune system, unable to challenge the progression and dissemination of the cancerous mass.

No data were obtained in the present work with nontransformed T cells of mucosal origin, given the paucity of the samples available. However, assuming that the HVS model confidently reflects the *in vivo* situation,[6] it seems adequate to assume that these proliferative defects would also be present in gastric T lymphocytes of patients with cancer.

Table 1 Main features of the HVS-derived T-cell lines used in this work

Cell line	*Patient/control*	*CD4/CD8*	*αβ/γδ*	*Culture time (months)*
G1HVS	P	CD4	αβ	>3
G2HVS	P	CD4	αβ	>3
G3HVS	P	CD4	αβ	>3
G4HVS1	P	CD8	αβ	>3
G4HVS2	P	CD8	αβ	>3
G5HVS	P	CD4	αβ	>3
G6HVS1	P	CD4	αβ	>2
G6HVS2	P	CD4	αβ	>2
C1	C	CD4	αβ	>3
C2	C	CD8	αβ	>3

METHODS

Gastric samples were obtained at surgery from six adult patients (five men, one woman, 60.3 years mean age, range 45–75 years) with gastric adenocarcinoma. Control specimens consisted of small intestine tissue samples obtained from two healthy age-matched individuals undergoing endoscopy for reasons other than cancer.

Tissue samples were processed as described previously[11] and the lymphocytes obtained were used for the transformation procedure.[6,11]

Once HVS-derived cell lines were established, they were stimulated with monoclonal antibodies to CD3 (α-CD3 1 μg ml^{-1}; Orthoclon OKT3, Ortho Biotech Products, Raritan, NJ, USA), α-CD2 (2.5 μg ml^{-1}; T11 1/1, clone 6G4,

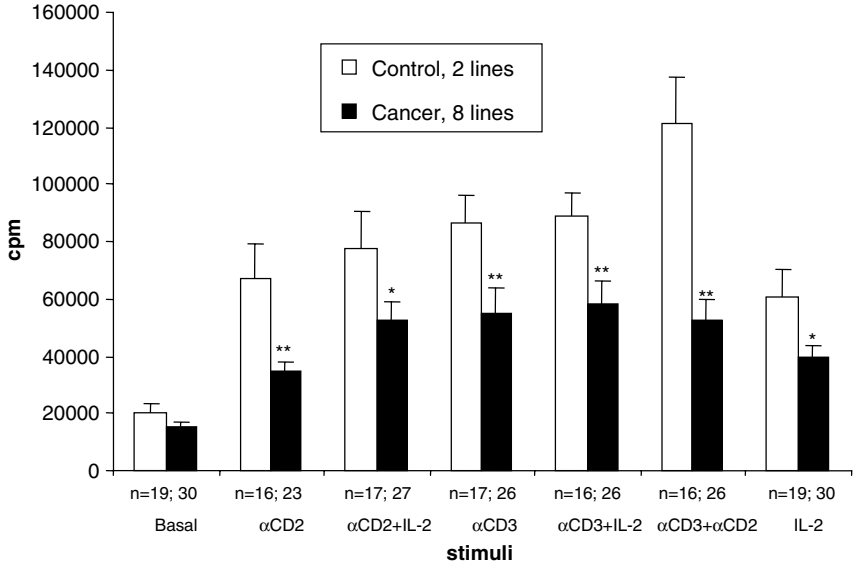

Figure 1 Proliferative response in counts per minute (c.p.m.) of *Herpesvirus saimiri* (HVS)-transformed T-cell lines of gastric origin. Eight gastric cancer-derived T-cell lines and two control lines were analysed. Average results obtained are shown. Cancer lines respond poorly when compared to control lines. Results are given as mean ± s.e. Only significant results are shown. See text for a complete list of stimuli used. *n* represents the number of times each stimulus was tested on the cell lines. *$P<0.05$, **$P<0.01$.

and 2/1, clone 4B2) or α-CD28 (50 ng ml^{-1}; CLB, Amsterdam, The Netherlands), alone or in combination with other mitogenic substances, such as IL-2, phorbol esters (phorbol 12-myristate 13-acetate, PMA, 10 ng ml^{-1} when used alone, 1.2 ng ml^{-1} when combined with ionomycin; Sigma Aldrich, St Louis, MO, USA) or ionomycin (ION 1 μM; Calbiochem, La Jolla, CA, USA).[6,12] Cell lines were left resting (deprived of IL-2) 24 h prior to stimulation.

Results obtained are shown as mean value ± s.e. of the mean (s.e.m.). Two-tailed Mann–Whitney two-sample test was used. Significance was reached when a *P*-value less than 0.05 was obtained.

All the experiments were carried out with the approval of the Ethics Committee of the institution.

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