γδ T cell non-responsiveness in *Campylobacter jejuni*–associated Guillain-Barré syndrome patients

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Abstract—To seek evidence for a role of molecular mimicry in the induction of Guillain-Barré syndrome (GBS), the authors studied Campylobacter jejuni–reactive T lymphocytes in patients with GBS. In contrast to controls, γδ T cells of patients with GBS with antecedent *C jejuni* infections failed to respond to *C jejuni*. Supplementing cell cultures with the cytokines interleukin-2 or interleukin-15 resulted in restoration of the γδ T cell proliferative response. γδ T cell non-responsiveness may lead to defective regulation of antibody production, and in this way an (auto)immune response against ganglioside-like epitopes on peripheral nerve may cause GBS.

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Guillain-Barré syndrome (GBS) is often preceded by *Campylobacter jejuni* enteritis. *C jejuni* lipopolysaccharides cross-react with ganglioside-like epitopes on peripheral nerve, suggesting that molecular mimicry plays a role in the induction of GBS. Whether a similar mechanism is active at the level of T cells, or what other role T cells may play, is unknown. We have recently shown that stimulation of peripheral blood mononuclear cells (PBMC) with *C jejuni* results in a strong expansion of γδ T cells in healthy individuals.[1] Here we analyzed the response of patients with GBS with and without antecedent *C jejuni* infection.

Methods. *Patients.* The characteristics of the patients are presented in the table. Serum from patients with GBS was tested for a recent infection with *C jejuni*, cytomegalovirus (positive for Patients 15 and 11), *Mycoplasma pneumoniae* (positive for Patient 20), and Epstein-Barr virus (all negative).[2] Patients with a history of *C jejuni* enteritis (positive *C jejuni* stool culture) were also included.

T-cell cultures and proliferation assays. Four strains obtained from the ATCC (ATCC 43429: serotype O:1; ATCC 43446: serotype O:19; ATCC 43445: serotype O:18; CCUG 10938: serotype O:4) and four isolated from stool of patients with GBS (O:1, O:2, O:19, and one untypable) were used. Heparinized blood from a patient with GBS and a healthy control are processed in parallel. PBMC were cultured with *C jejuni* sonicate at a concentration of 3 μg protein/mL for 12 days prior to cell counting and flow cytometric analysis, or 5 days prior to a proliferation assay.[1] Interleukin (IL)-2 (Strathmann Biotech, Hannover, Germany) and IL-15 (Pepro Tech, Rocky Hill, NJ) were used at 10 U/mL and 10 ng/mL.

Antibodies. Anti-CD3-PECy5, goat anti-mouse-PE, anti-FasL, anti-TCR ζ chain, and anti-Fas-Fitc were obtained from Immunotech (Fullerton, CA). Anti-TCR Vδ1 (clone A13) was a gift of Dr. L. Moretta, Genova; anti-TCR Vδ2 (clone 4G6), anti-Vγ4 (clone 4A11), and anti-Vγ9 (clone B3) were gifts of Dr. G. De Libero, Basel; anti-CD94 was a gift of Dr. M. Lopez Botet, Madrid; and all other antibodies were from Becton Dickinson. Stainings and analysis were performed as described.[1]

Statistical analyses. The mean of triplicate measurements of ^{3}H thymidine counts and expansion indices was log-transformed prior to statistical evaluation. Values obtained from culture medium control stimulations were subtracted from experimental values. A two-tailed Mann-Whitney rank sum test was used to analyze differences between groups.

Results. Results from stimulations with different *C jejuni* strains were similar and therefore averaged. PBMC of 30 healthy donors vigorously proliferated upon stimulation with *C jejuni* sonicate (median 30×10^3 cpm, range 7.6 to 101×10^3 cpm; figure 1A). In contrast, PBMC of six patients with GBS with preceding *C jejuni* infection did not proliferate upon stimulation with *C jejuni* sonicate (median 1.2×10^3 cpm, range 0.12 to 3.2×10^3 cpm; figure 1B) ($p < 0.001$). PBMC of patients with GBS without *C jejuni* infection proliferated similar to healthy individuals (median 48×10^3 cpm, range 4.6 to 139×10^3 cpm).

To assess whether the observed non-responsiveness was transient, we analyzed blood of Patient 1 2 months later and of seven patients who had a history of *C jejuni*–related GBS. The response in these patients was decreased compared to healthy controls (median 3.9×10^3 cpm, range 0.2 to 47×10^3cpm; $p < 0.01$; see figure 1B). Two of the eight recovered patients responded similarly to the healthy individuals. Control patients with a history of uncomplicated *C jejuni* enteritis showed high responses (median 52×10^3 cpm, range 32 to 68×10^3 cpm; see figure 1B) and differed from the group of recovered *C jejuni*–positive patients with GBS ($p < 0.01$).

Flow cytometric analysis and calculation of the expansion index of αβ and γδ T cells demonstrated strong selective expansion of CD3+/TCRγδ+ T cells in the PBMC cultures that showed a significant proliferative response (not shown). The frequency and Vγ/Vδ utilization of γδ T cells was analyzed, and double stainings with a pan-γδ marker in combination with monoclonal antibodies against

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Table *Characteristics of the GBS and* Campylobacter jejuni *enteritis patients*

Patient	Time after onset	Sex	Age at onset, y	Severity score	Prodromal disease	*C jejuni*	3H counts ($\times 10^3$)
Acute GBS							
1	Acute	M	57	5	GI	+	0.1
2	Acute	F	36	4	GI	+	1.1
3	Acute	M	46	3	GI	+	1.2
4	Acute	F	54	4	GI	+	1.2
5	Acute	M	58	3	GI	+	2.8
6	Acute	F	82	4	No	+	3.2
7	Acute	M	46	4	UR	−	4.6
8	Acute	F	59	2	No	−	21.8
9	Acute	M	45	4	No	−	48.4
10	Acute	F	49	3	UR	−	70.9
11	Acute	F	33	3	UR	−	138.7
Recovered from GBS							
1	2 mo	M	57	5	GI	+	0.2
12	3 y	M	58	5	GI	+	0.9
13	7 y	M	38	5	GI	+	1.1
14	3 y	M	64	3	GI	+	3.7
15	3 y	F	33	2	GI/UR	+	4.1
16	7 y	M	44	5	GI	+	6.9
17	3 y	F	44	2	GI	+	24.7
18	3 mo	F	31	3	GI/UR	+	46.7
19	7 y	F	58	4	UR	−	4.6
20	3 mo	F	23	3	UR	−	16.3
21	4 y	M	56	4	UR	−	33.3
11	7 mo	F	33	3	UR	−	40.2
Recovered from *C jejuni* enteritis							
22	4 y	F	70		GI	+	32.1
23	2 y	M	31		GI	+	35.7
24	2 y	F	50		GI	+	52.1
25	1 y	M	43		GI	+	60.9
26	3 y	M	58		GI	+	68.2

The period between onset of GBS or *Campylobacter jejuni* enteritis and inclusion in our experiments is depicted as time after onset of disease. Severity of GBS was scored at the peak of illness on a functional disability scale on which 0 denotes healthy; 1, minor symptoms; 2, able to walk >10 m without assistance; 3, able to walk >10 m with support; 4, bedridden or chairbound; 5, requiring assisted ventilation for at least part of the day; 6, dead. Antibodies against cytomegalovirus, Epstein-Barr virus, and mycoplasm were not determined in *C jejuni* enteritis patients.

GBS = Guillain-Barré syndrome; GI = gastrointestinal; UR = upper respiratory.

molecules that provide information on activation status (HLA-DR), anatomic origin (CD8), TCR-mediated signal transduction capacity (TCRζ chain, CD3, and FcRIII), apoptosis related molecules (Fas/FasL), or killer cell inhibitory receptors (CD94) were performed. No significant difference between GBS and healthy control groups was found.

Flow cytometric analysis and counting of *C jejuni*–stimulated cells at 24-hour intervals unveiled unaltered absolute numbers of γδ T cells within the CD3+ population during the first 7 days in culture, showing that γδ T cells of non-responding patients with GBS did not die upon stimulation. The basis for γδ T cell non-responsiveness was further investigated by adding cytokines to the cell cultures. We recently showed that expansion of γδ T cells from healthy donors upon stimulation with *C jejuni* is dependent on the presence of CD4+/αβ+ T cells in cultures or addition of exogenous IL-2 or IL-15.[1] To study the role of IL-2 and IL-15, PBMC of four non-responding patients with GBS were stimulated with *C jejuni*, IL-2, IL-15, or with *C jejuni* in combination with these cytokines. After 12 days of culture, expansion indices of γδ T cells were determined. Figure 2 shows that γδ T cells from *C jejuni*–associated patients with GBS do not respond to stimulation with any of the single compounds, but that

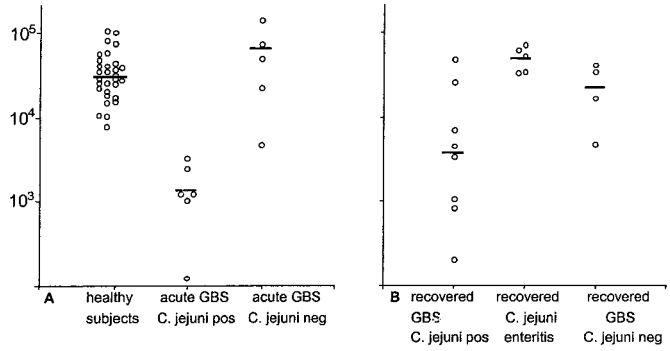

Figure 1. Proliferative responses of peripheral blood mononuclear cells (PBMC) to Campylobacter jejuni *antigen. Proliferative response to* C jejuni *antigens of healthy donors,* C jejuni*–induced patients with Guillain-Barré syndrome (GBS), and patients with GBS without preceding* C jejuni *infection (A), and patients with a history of* C jejuni *enteritis–induced GBS, uncomplicated* C jejuni *enteritis, or* C jejuni*–unrelated GBS (B). Incorporated ^3H thymidine counts are means of triplicate wells of stimulations with different bacterial strains.*

stimulation with *C jejuni* in combination with IL-2 or with IL-15 results in a strong proliferation, comparable to that observed with PBMC from normal donors.

Discussion. γδ T cells in patients with GBS with an antecedent *C jejuni* infection fail to proliferate after stimulation with this microorganism, in contrast to a vigorous *C jejuni*–induced response in healthy individuals, individuals with *C jejuni*–related acute enteritis, and patients with GBS without evidence of an antecedent *C jejuni* infection. These differences, and our finding that the *C jejuni* strains isolated from patients with or without GBS did not differ in their capacity to stimulate the proliferation of γδ T cells, suggest that T cell reactivity is more likely to be influenced by host factors than by bacterial strain differences.

Contrary to our findings, others described a stronger proliferative response in patients with GBS than in healthy donors.[3] *C jejuni*–reactive clones that we derived from healthy donors have the Vδ2/Vγ9 TCR and react to phosphoantigen.[1] Our antigen preparations may contain relatively high concentrations of phosphoantigens, to which healthy donors are responsive and patients with GBS are non-responsive, and the antigen preparations used by others may contain more of another antigen that stimulates phosphoantigen unresponsive Vδ1 cells. Our results are consistent with observations that patients with GBS are unresponsive to the phosphoantigen isopentenylpyrophosphate.[4]

We observed that in some patients γδ T cell non-responsiveness lasted for prolonged periods of time after clinical recovery. In the most extreme case, the peripheral blood γδ T cells of two patients who had recovered from *C jejuni*–related GBS 7 years before the time of the experiment still failed to respond.

We did not detect quantitative or phenotypic differences in the γδ T cell population in patients with GBS and healthy controls that could explain the non-responsiveness. However, γδ T cell non-responsiveness could be restored by simultaneous stimulation with *C jejuni* and the cytokines IL-2 or IL-15, indicating that deficient production of cytokines, and not an inherent property of γδ T cells, leads to γδ T cell non-responsiveness. These cytokines are indeed driving cultures of healthy donor PBMC stimulated with *C jejuni*, because we have recently shown that blocking antibodies against these cytokines can inhibit the γδ T cell response.[1] Furthermore, low production of IL-2 upon polyclonal T cell activation and impaired responses to IL-2 have been reported in GBS.[5]

γδ T cells have shown to alter antibody responses in transgenic mice.[6,7] In GBS, antibodies against *C jejuni* cross-react with peripheral nerve tissues. The concomitant lack of cytokine production upon *C jejuni* stimulation and ensuing γδ non-responsiveness may result in defective regulation of autoantibody production and disease.

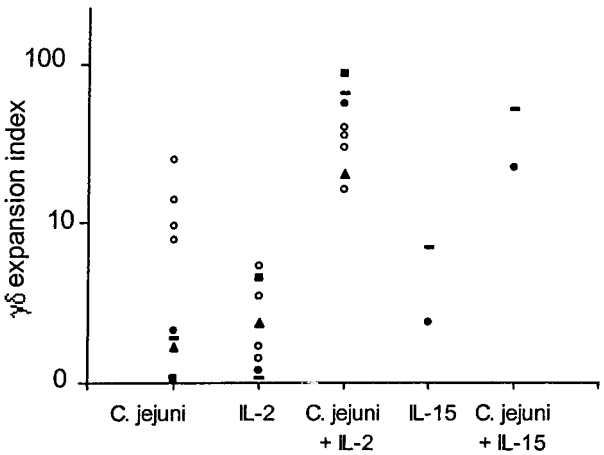

Figure 2. Restoration of the γδ T *cell proliferative response by cytokines. Response of healthy donors (open circles) and* Campylobacter jejuni *non-responsive patients with Guillain-Barré syndrome (GBS) (each patient represented by a different black symbol) to* C jejuni*, interleukin (IL)-2 or IL-15 alone, and* C jejuni *antigen in combination with IL-2 or IL-15. The γδ T cell expansion index is plotted on the y-axis.*

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