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To the editor:

Maintenance of HCV-specific T-cell responses in antibody-deficient patients a decade after early therapy

Early therapy for hepatitis C virus (HCV) is associated with a high rate of viral clearance, but the long-term effects on immune responses remain controversial. The role of antibodies, both acutely and in the long term, is not clearly defined. We investigated these issues in a unique cohort of 7 individuals with primary antibody failure, who had received early interferon therapy after infection through contaminated immunoglobulin therapy a decade previously.

In 1994, an outbreak of hepatitis C virus infection, genotype 1a, occurred in 30 hypogammaglobulinemic patients in the United Kingdom from 1 batch of contaminated immunoglobulin. Treatment with interferon (IFN)–α was initiated within 6 months of inoculation, as reported previously.[1,2] We were able to study 7 patients who had survived for 10 years, 5 remaining polymerase chain reaction–negative (PCR^-) many years after treatment (or spontaneous resolution in 2 cases) and 2 who were still PCR^+.

T-cell responses in peripheral blood were first studied using enzyme-linked immunospot assays with peptide pools spanning the whole HCV genome arranged in a matrix (described as matrix ELISpot), as well as specific human leukocyte antigen (HLA)–A2– and HLA-A1–restricted peptides.[3] Incorporation of ^{3}H-thymidine and CFSE (5,6-carboxylfluorescein diacetate succinimidyl ester; Molecular Probes, Eugene, OR) proliferation assays were performed, using recombinant HCV proteins NS3, NS4, and NS5 (Chiron, Emeryville, CA); HCV core peptide pools; tetanus toxoid (TT); or cytomegalovirus (CMV) lysate as previously described.[4,5]

Matrix ELISpot analysis revealed responses in 6 of 7 HCV-exposed patients (Figure 1A), but 0 of 5 hypogammaglobulinemic HCV-PCR^- controls (P = .015). Strong HCV-specific responses were detected in all 5 HCV-exposed PCR^- patients (Figure 1A-B) and 1 of 2 PCR^+ patients.

$CD8^+$ T-cell responses were examined using class I–restricted peptides in IFN-γ ELISpot. These were demonstrated in all patients tested (2 HLA-A2 and 1 HLA-A1; Figure 1A-B). We also analyzed HCV-specific $CD4^+$ T-cell populations using a fluorescence-activated cell sorting (FACS)–based assay (Figure 1A,C), revealing substantial proliferation in 5 of 7 individuals. Responses to NS3-5, characteristic of immunocompetent spontaneous resolvers, were observed in 4 of 5 PCR^- patients, but 0 of 2 PCR^+ patients, and confirmed using conventional tritium assays.

Here we have shown a sustained response to antiviral therapy in acute HCV in the absence of specific antibody, associated with T-cell responsiveness maintained over a decade. Such activity was specific, was detected using a variety of methods, and included $CD8^+$ and $CD4^+$ T-cell responses. Two patients in the group did

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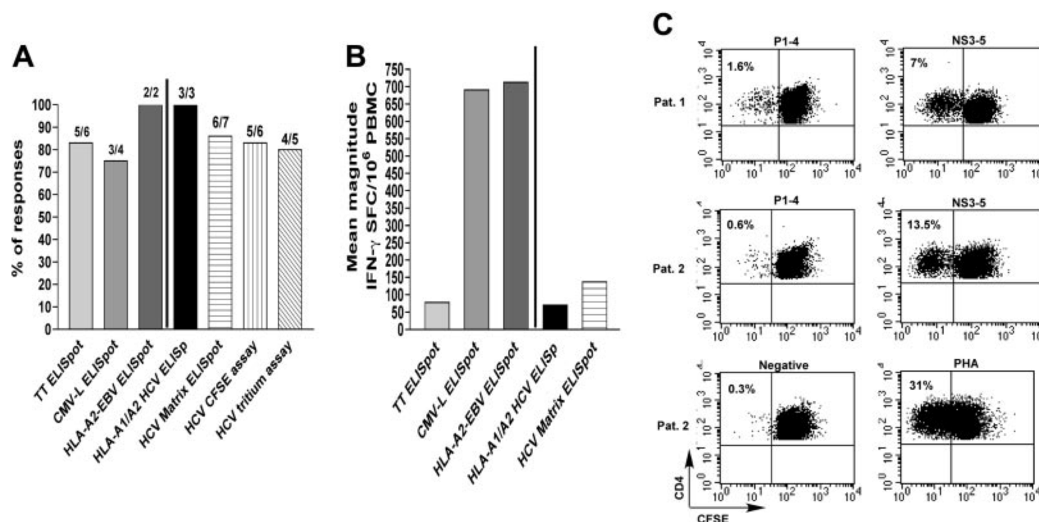

Figure 1. Analysis of HCV-specific and other T-cell responses in hypogammaglobulinemic cohort. (A) Percentage of positive responses to different antigens tested using a range of techniques. Numbers on top of each bar show absolute numbers of responding individuals tested for the various antigens. The numbers include those tested from the whole cohort, both PCR^+ and PCR^-. Matrix ELISpot indicates antigens tested were 58 pools containing 301 overlapping peptides (P1-P58) spanning the whole HCV genome; TT, tetanus toxoid, CMV-L, cytomegalovirus lysate; A2-EBV, Epstein Barr virus peptide HLA-A2–GLCTLVAML, A2-HCV, HCV peptide HLA-A2–CINGVCWTV; and A1 HCV, HCV peptide HLA-A1–ATDALMTGY. (B) Mean magnitude of antigen-specific T-cell responses in ELISpot. Magnitude is shown as IFN-γ spot-forming cells (SFCs)/10^6 peripheral blood mononuclear cells (PBMCs). For matrix ELISpot, mean number of reactive pools is 11 (range, 4-26 pools); mean magnitude is 137 SFC/10^6 PBMCs (range, 45-360 SFC/10^6 PBMCs). (A-B) The vertical line divides each graph into control antigens (left) and HCV antigens (right). (C) HCV-specific $CD4^+$ proliferative responses as determined by the CFSE assay. Results are shown for 2 PCR^- hypogammaglobulinemic individuals previously exposed to HCV (first patient in top panels; second patient in middle and bottom control panels). PBMCs were stimulated for 6 days using HCV core peptide pools and HCV nonstructural proteins NS3/4 and NS5 as described. Responses represent the percentage of proliferating $CD4^+$ T cells after subtraction of the background (negative). Undivided $CD4^+$ T cells are detected in the top right quadrants of each FACS plot, and the CFSE signal is diluted with each cell division as the dye is distributed to the daughter cells. Numbers in the top left quadrants of each plot represent the percentage of HCV peptide– or protein-specific $CD4^+$ T cells that have proliferated during the 6-day culture. One negative and 1 positive (phytohemagglutinin [PHA]) control are shown in the bottom panel. Cells are gated on $CD4^+$ and Viaprobe-negative (live) cells (Viaprobe; Becton Dickinson, San Diego, CA).

not receive therapy because they controlled the infection spontaneously, and these showed comparable responses in ELISpot and proliferation assays. Thus, the responses seen in these antibody-deficient patients who were IFN-α treated are not substantially different from untreated, spontaneously resolved, antibody-deficient individuals, or other spontaneous resolver cohorts,[3,4] although a much larger cohort would be needed to assess this definitively.

The role of antibodies in long-term antiviral T-cell memory in humans is not known, although it plays a critical role in murine models. Single-source outbreaks of HCV have been highly informative as to the natural history of HCV infection and long-term outcomes of early treatment. Here, such an outbreak provides a unique insight into the longevity of T-cell responses in the absence of antibodies.

Nasser Semmo, Michaela Lucas, George Krashias, Georg Lauer, Helen Chapel, and Paul Klenerman

Correspondence: Paul Klenerman, The Peter Medawar Building for Pathogen Research, University of Oxford, South Parks Road, Oxford OX1 3SY, United Kingdom: e-mail: paul.klenerman@medawar.ox.ac.uk.

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To the editor:

Complete regression of cutaneous lesions of refractory Ph+ ALL after 4 weeks of treatment with BMS-354825

Imatinib (Glivec; Novartis, Basel, Switzerland), specifically inhibiting ABL kinase, has evidenced an important role in chronic myeloid and Philadelphia chromosome–positive (Ph^+) acute lymphoblastic leukemias (ALLs); however, resistance is increasingly encountered,[1,2] primarily mediated by mutations within the kinase domain of BCR-ABL that interfere with drug binding.[3,4]

The second generation of tyrosine kinase inhibitors, which include BMS-354825 (Dasatinib; Bristol Myers Squibb, Princeton, NJ), also target ABL,[5] but they appear able to bind also the majority of mutated forms of the protein.[6]

We present the case of a patient with relapsed/refractory Ph^+ ALL with disease at extramedullary (cutaneous) and blood level treated with BMS-354825 who achieved complete response in a very short period of time. This 67-year-old male patient was diagnosed with Ph^+ ALL (70% Ph^+ metaphases, p190 transcript) $CD10/CD19/CD38^+$, in September 2002. He was treated with

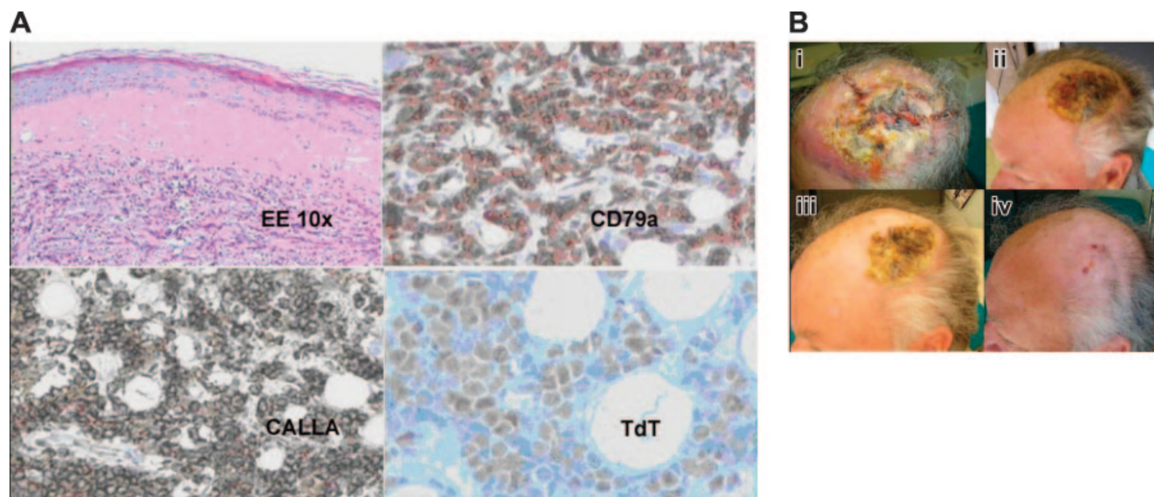

Figure 1. Cutaneous response to BMS-354825. (A) Skin biopsy shows cutaneous involvement by precursor B cells; neoplastic cells diffusely infiltrate the dermis. Hematoxylin-eosin staining. Low magnification, × 10. At immunohistochemistry, the lymphoblasts express B-cell markers as CD79a, precursor B-cell marker CD10-CALLA, and show nuclear staining for TdT. Images were obtained with a Nikon Eclipse E600 microscope (Nikon, Tokyo, Japan) with 10 ×/0.25 NA and 40 ×/0.65 NA brightfield objectives, acquired with a JVC TK 1270 color video camera (JVC, Tokyo, Japan), and AVer E-Z Capture software (AVerMedia, Milpitas, CA). Images were processed with Paint Shop Pro 7.0 (Jase Software, Eden Prairie, MN). CD79a, CALLA, and TdT are from Dako (Glostrup, Denmark). (B) Patient with cutaneous infiltrate of acute lymphoblastic leukemia resistant to previous treatments. In panel Bi, presence of multiple dermohypodermal nodules with a central ulceration pre–BMS-354825 therapy. In panels Bii-iii the same lesion 1 and 2 weeks after starting therapy. Four weeks after treatment, the scalp lesion was completely healed with only the presence of a hypopigmentated scar (Biv). Pictures were taken using a Panasonic Lumix 2MC-FX5 (Panasonic, Osaka, Japan) digital camera and processed with Paint Shop Pro 7.0.

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