Multiple Modes of Interaction between Lck and CD28

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LETTERS TO THE EDITOR

J_H6 Gene Usage among HCV-Associated MALT Lymphomas Harboring t(14;18) Translocation

In the September 1, 2004, issue of *The Journal of Immunology*, Sasso et al. (1) demonstrated an increased frequency of J_H6 gene usage among t(14;18) translocations in B cells from hepatitis C virus (HCV)-infected individuals. We have recently detected *bcl-2* rearrangement in lymphomas of MALT from HCV-infected patients (2). On the basis of the data reported by Sasso et al. (1), we sought to investigate which J_H genes were preferentially used in our series. Nucleotide sequence analysis revealed that *bcl-2* was joined to J_H6 in all four HCV-associated MALT lymphomas with t(14;18) translocation (see Table I). Our data, supporting the findings by Sasso et al. (1), add that *bcl-2* is frequently fused to J_H6 in MALT lymphomas from HCV-infected patients. Additional studies are needed to better characterize the role of t(14;18) in lymphomagenesis among HCV-infected individuals.

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The Authors Respond

The finding by Libra et al. (1) that the t(14;18) in each of four HCV-associated MALT lymphomas used a J_H6 gene is consistent with our finding that the t(14;18) found in PBMC of HCV^+ subjects are strongly biased to use J_H6 genes (2). The authors have previously reported finding t(14;18) in HCV-associated MALT lymphomas (3). An association has not been established, however, between MALT lymphomas and HCV infection or between MALT lymphomas and t(14;18). A better understanding of these areas will help place the present finding in proper context.

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Multiple Modes of Interaction between Lck and CD28

In a recent paper, Tavano et al. (1) reported the essential role of a C-terminal ^{208}PXXPP^{212} motif in CD28 for the recruitment of Lck to the immunological synapse, and we would like to comment on their results in the light of the affinity of different CD28-Lck interactions that we have studied in vitro by biophysical methods.

Peptides comprising residues 206–220 of human CD28 and 205–215 of murine CD28, respectively, bind to the SH3 domain of Lck only with very low affinity ($K_d > 1$ mM). Although

Table I. *DNA sequences at the bcl-2/J_H breakpoint of PCR products amplified from all four HCV-associated MALT lymphoma biopsy specimens*[a]

Patient	*bcl-2* Nucleotides	N Segment	J_H Nucleotides	J_H Gene
1	ccagacctccccggcgggcc-3170	ggcccctcgggaaactcccgtatagtggctacgattcc	1481-ttactactactactacggta	J_H6
3	ccttccagggtcttcctgaa-3098	gggtaagcgccttctatggcgc	1480-tttactactactactacggt	J_H6
6	caccaagaaagcaggaaacc-3138	atataggatgtttggagtgtgctacgg	1475-tggaatttactactactact	J_H6
9	tcctgaaatgcagtggtgct-3112	ccctggttccccgaa	1482-tactactactactacggtat	J_H6

[a] These PCR products were obtained after both first and second rounds of amplification from tumors exhibiting *bcl-2* rearrangement. Nucleotide numbering corresponds to that of GenBank accession numbers M14745 and M25625 for *bcl-2* and the J_H gene segment of IgH, respectively.

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we agree that this interaction can nevertheless be of physiological relevance in a situation in which both proteins are membrane associated and located in lipid rafts, we want to draw attention to an alternative CD28 interaction that can be formed after phosphorylation of Tyr^{209} of human CD28 with the Lck SH2 domain (Fig. 1). This interaction (K_d = 2.13 μM) is in vitro at least three orders of magnitude stronger than the SH3 interaction and is in the range of those observed for other SH2-ligand interactions (2).

Evidence that such a CD28-Lck interaction may also play a role in vivo comes from previous studies of Sadra et al. (3), who showed that the corresponding tyrosine (Tyr^{188} in their study) becomes phosphorylated after stimulation of murine CD28 expressed in Jurkat cells. Moreover, a Tyr→Phe mutation at this position severely impaired the ability of mCD28 to deliver a costimulus for the expression of CD69 and the production of IL-2 (3). Although tyrosine phosphorylation at this position could not be associated with any specific signaling event up to now, our data suggest that this residue is likely involved in Lck SH2 binding. We feel that this finding has important implications for the design of new experiments that should also take into account the existence of a second mode of CD28-Lck interaction involving the SH2 domain in addition to the SH3 interaction reported by Tavano et al. (1).

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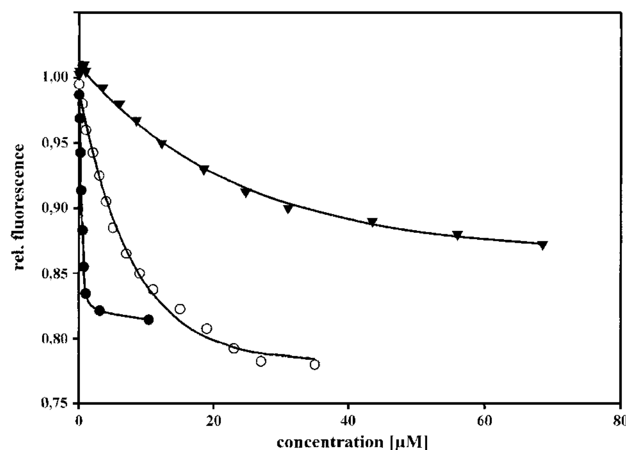

FIGURE 1. Affinity of phosphorylated $hCD28_{206-220}$ (○) for the Lck SH2 domain: Changes of the relative fluorescence were measured in a competitive binding study that was performed as described in Ref. 2. $hCD28_{206-220}$ binds with a K_d of 2.13 μM and thus with higher affinity to the SH2 domain than the phosphorylated autoinhibitory Lck C terminus (▼) (K_d = 8.99 μM). Titration curves for a high-affinity SH2 ligand (●) containing a YEEI binding motif (K_d = 0.10 μM) and for the Lck C terminus were reprinted with permission from Ref. 2 and are shown for comparison.

The Authors Respond: Multiple Models of Interaction between Lck and CD28

In our recent paper titled "CD28 and Lipid Rafts Coordinate Recruitment of Lck to the Immunological Synapse of Human T Lymphocytes," we demonstrated the essential role of the C-terminal ^{208}PXXPP^{212} motif in CD28 for the recruitment of lipid rafts, and therefore of the raft-associated protein Lck, to the immunological synapse (IS) (1). We do not believe that the lower recruitment of Lck to the IS of T cells expressing the CD28-3A mutant is the consequence of a lower affinity of the CD28-Lck interaction. First, we demonstrated that not only Lck but also a myristoylated and palmitoylated fluorescent protein, used as raft marker, is less recruited to the IS of CD28-3A-expressing T cells (Fig. 2 of our paper). Second, in agreement with the model proposed by Hofinger and Sticht, we have data indicating that the direct interaction between CD28 and Lck does not require the CD28 C-terminal ^{208}PXXPP^{212} motif (our manuscript in preparation).

Our paper indicates that a large fraction of Lck is recruited to the IS of T cells engaging a $B7^+$ APC as a result of lipid rafts mobilization, an event requiring CD28 signaling through its C-terminal ^{208}PXXPP^{212} motif.

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