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# Heterogeneity of CD4-Positive Human T-Cell Clones Which Recognize the Surface Protein Antigen of *Rickettsia typhi*

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**Immunity to the typhus group of rickettsiae is largely dependent on the effector function of several classes of T lymphocytes, including those which produce gamma interferon. Since the surface protein antigen (SPA) derived from typhus group rickettsiae has been shown to be an effective immunogen in animal models, human T-cell clones specific for the SPA of *Rickettsia typhi* were isolated and tested for their antigenic specificity, as well as for their ability to produce gamma interferon. Eighteen CD4-positive clones specific for the SPA of *R. typhi* exhibited considerable diversity in their response to the SPAs derived from two strains of *Rickettsia prowazekii* and from *Rickettsia canada*. The vast majority of clones also recognized the SPAs from *R. prowazekii* but not from *R. canada*. Two heteroclitic clones demonstrated significantly higher proliferative responses to the SPAs derived from one or both of the *R. prowazekii* strains than to the SPA of *R. typhi*, and one clone demonstrated a significantly higher response to the SPA of *R. typhi* than to the other SPAs. All 18 clones produced gamma interferon in response to SPA stimulation. We conclude that the SPAs from typhus group rickettsiae can elicit both a diverse T-cell response in humans and the efficient stimulation of gamma interferon-mediated immunity.**

The immunologic responses of nonprimate and primate hosts to typhus group rickettsiae have been studied extensively. In animal models, protection against virulent organisms appears to be largely T lymphocyte dependent (7), and in humans a variety of cell-mediated mechanisms whose goal is to bring about the lysis of the infected host cell also appear to be operative (4, 5). Gamma interferon has been shown to cause the lysis of cells infected with typhus group rickettsiae as well as to inhibit their intracellular growth (16, 17).

Since the species-specific surface protein antigens (SPAs) of both the typhus group rickettsiae (2) and the spotted-fever group (14) are capable of inducing protective immunity in animal models, it is of major interest, before using the SPAs as possible subunit vaccines in humans, to know whether this unique class of proteins is also capable of triggering any of the human cellular immune mechanisms previously described. In the present study, 18 human CD4-positive lymphocyte clones which specifically recognized the SPA of *Rickettsia typhi* were heterogeneous in their recognition of the SPAs derived from four different species or strains of typhus group rickettsiae. All 18 clones produced significant amounts of gamma interferon. These observations thus confirm the heterogeneity of the SPA from different species or strains of typhus group rickettsiae, illustrate the diversity possible in the human T-cell response to a bacterial protein antigen, and demonstrate that the SPA is important in eliciting gamma interferon-mediated immunity. Most important, however, we believe these results point to the usefulness of the SPAs as protective vaccines against typhus in humans.

## MATERIALS AND METHODS

**Antigen activation and cloning of PBMC.** Peripheral blood mononuclear cells (PBMC) obtained from one individual who had both historic and serologic evidence of previous infection with *R. typhi* were stimulated in vitro with *R. typhi* SPA for 7 days as already described (4). Following the 7-day stimulation, blast cells obtained by Ficoll-Hypaque centrifugation were cloned at 0.3 cell per well in sterile 60-well Terasaki plates in the presence of T-cell growth factor (12), autologous irradiated (4,000 rads of $^{137}Cs$) PBMC, and water-extracted SPA derived from *R. typhi* at a concentration of 2.5 μg/ml. Cloning efficiency was determined to be 3.5%, and therefore, according to a proposed mathematical model (9), the probability was 99.2% that the contents of wells demonstrating cell growth represented the clonal expansion of a single cell. The contents of each well demonstrating cell growth will therefore be referred to as a clone in this paper. Cells from expanding clones were then tested for the ability to proliferate in response to SPA in the absence of T-cell growth factor and frozen to be expanded and used in future experiments.

**Antigenic specificities and phenotypic characterization of the clones.** Cloned cells were stimulated with water-extracted SPAs of *R. typhi* (Wilmington strain), *Rickettsia prowazekii* (Breinl and Madrid E strains), or *Rickettsia canada* (McKiel strain) at a concentration of 2.5 μg/ml, and proliferation was measured by the incorporation of [$^3H$] thymidine (12). High-performance liquid chromatography-purified (11) SPA antigens were used in dose dependence experiments at concentrations of 1, 2.5, 5, 10, and 20 μg/ml. Five replicates for each group were set up. Clones were phenotyped by flow cytometry (1).

**Gamma interferon determinations.** Prior to harvesting [$^3H$] thymidine-pulsed cells onto glass fiber filters, 100 μl of supernatant from each well was removed and stored at −20°C. Levels of gamma interferon in these supernatants were then determined either by biological assay (13) or by radioimmunoassay (RIA) (Gamma-Interferon RIA; Centocor Inc., Malvern, Pa.). The maximum detectable level of gamma interferon was approximately 50 IU/ml as determined by RIA. Samples with greater than 50 IU/ml were

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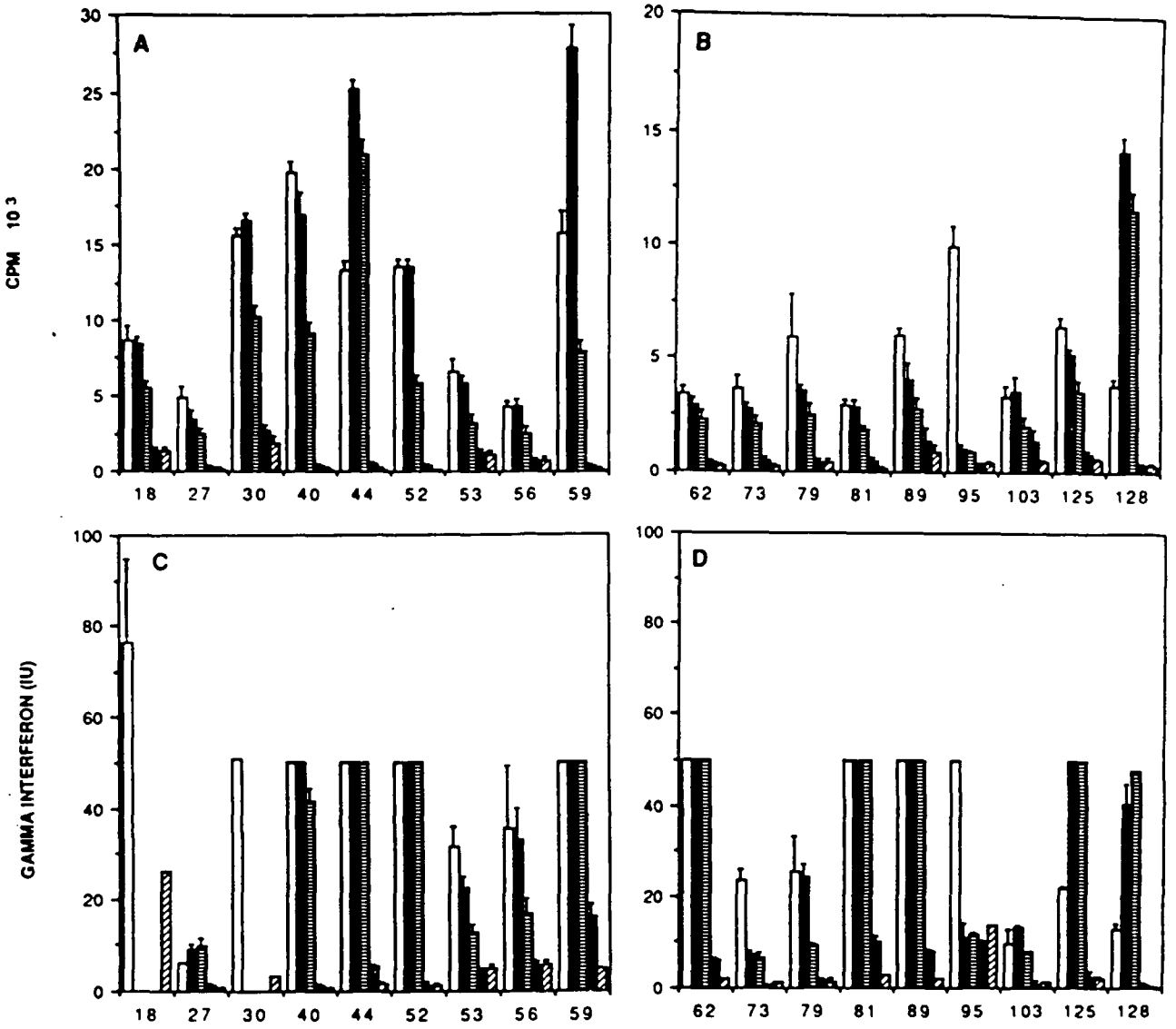



FIG. 1. (A and B) Eighteen CD4-positive clones specific for the SPA of *R. typhi* were stimulated with water-extracted SPAs (2.5 μg/ml) derived from (bars from left to right) *R. typhi* (Wilmington strain, □), *R. prowazekii* (Breinl strain, ■), *R. prowazekii* (Madrid E strain, ▣), or *R. canada* (McKiel strain, ▩) or with medium alone (⊡). Bars for each clone represent the mean and standard deviation for two to five experiments, each experiment consisting of five replicates. (C and D) Gamma interferon levels in culture supernatants were determined by either biological assay (clones 18 and 30) or RIA in response to stimulation with water-extracted SPAs (at a concentration of 2.5 μg/ml) derived from (bars from left to right) *R. typhi* (Wilmington strain), *R. prowazekii* (Breinl strain), *R. prowazekii* (Madrid E strain) or *R. canada* (McKiel strain) or with medium alone (symbols shown above). Bars for each clone represent the mean and standard deviation for two replicates. Culture supernatants were obtained from a representative experiment for each clone.

reported as such since limited amounts of supernatant prevented dilution and retesting.

## RESULTS

**Generation of SPA-specific T-cell clones.** PBMC stimulated with SPA derived from *R. typhi* in four separate bulk cultures were cloned by limiting dilution into a total of 5,400 wells. Of 194 growing clones, 19 (9.7%) were found to be antigen specific. Eighteen of these clones were found to be CD3,4 positive, whereas the remaining clone was found to be CD3,8 positive.

**Antigenic specificities of the 18 CD4-positive clones specific for SPA derived from *R. typhi*.** The ability of the 18 CD3,4 antigen-positive clones to proliferate in the absence of TCGF in response to the SPAs mentioned previously or to a medium control is summarized in Fig. 1A and B. With the exception of clone 95, all clones exhibited significantly higher responses to the SPAs of *R. typhi* and the two *R. prowazekii* strains than to the medium control ($P \leq 0.05$, Student's $t$ test). None of the clones demonstrated significant proliferation in response to the SPA of *R. canada* compared with the medium control ($P > 0.05$). The prolifer-

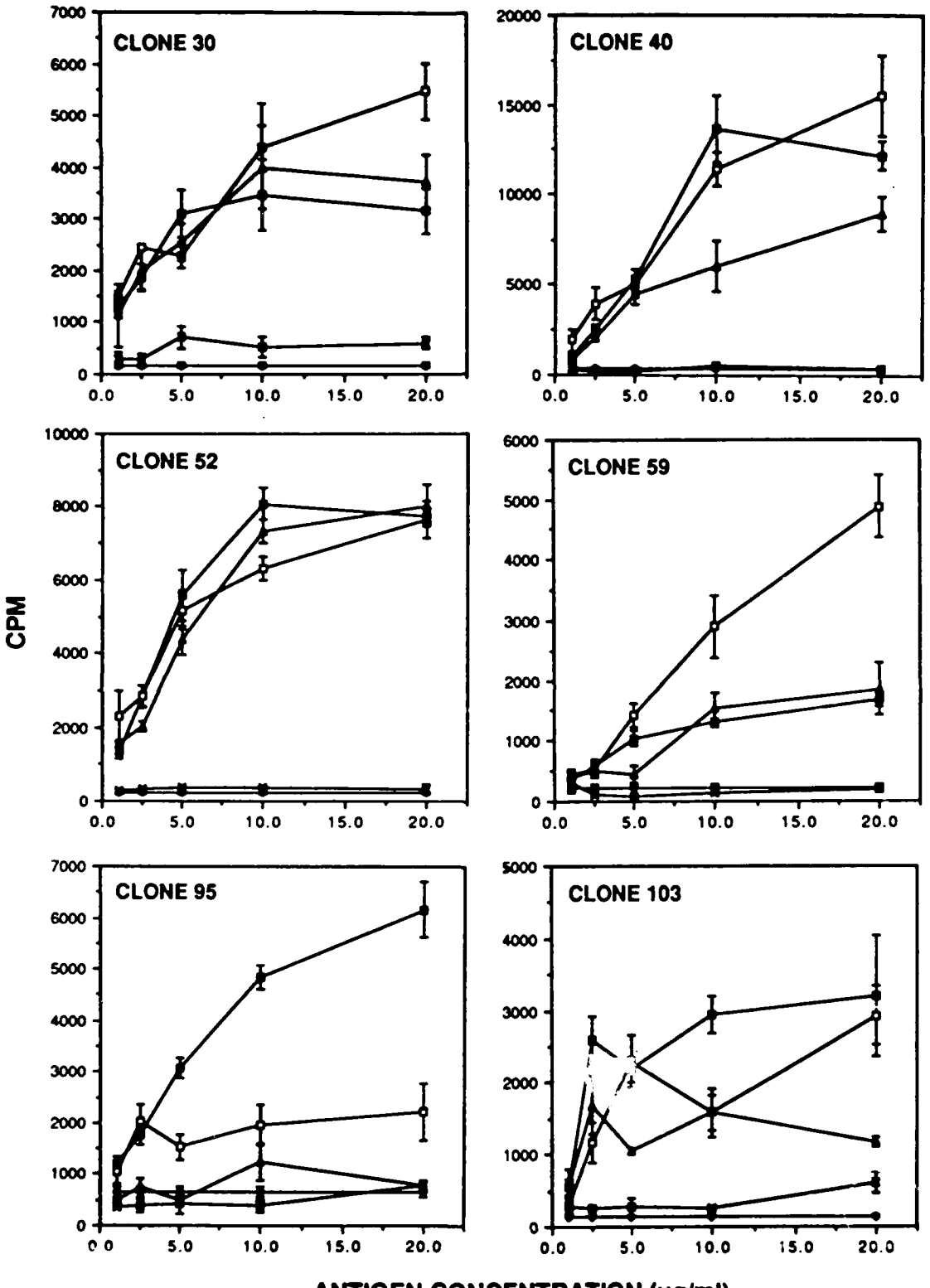
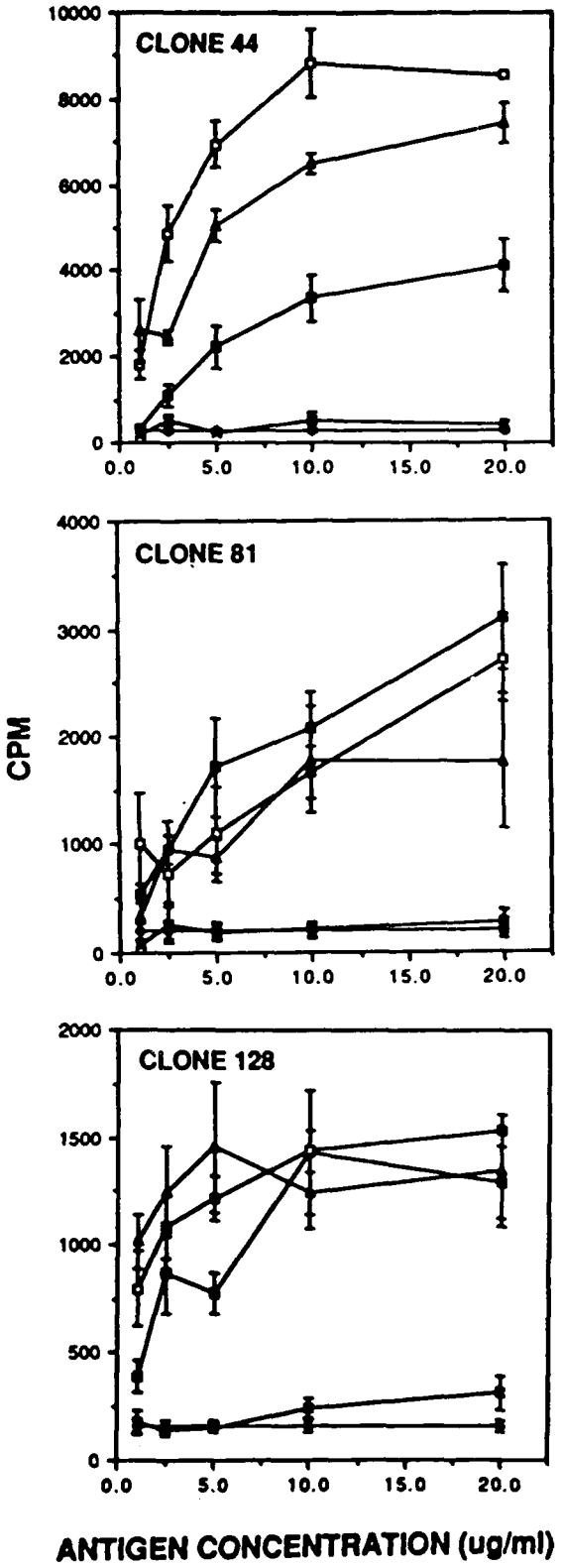



FIG. 2. Ten SPA-specific clones were stimulated with the high-performance liquid chromatography-purified SPAs of *R. typhi* (Wilmington strain, ■), *R. prowazekii* (Breinl strain, □), *R. prowazekii* (Madrid E strain, ▲), or *R. canada* (McKiel strain, ×) or with a medium alone (◆), with concentrations of SPA ranging from 1 to 20 μg/ml. Each point represents the mean and standard deviation for five determinations.




ative response of three of the clones (59, 44, and 128) was significantly higher ($P \leq 0.05$) in response to one or both of the SPAs from *R. prowazekii* than to the SPA derived from *R. typhi*, thus suggesting the heteroclitic nature of these clones. In addition, clone 95 proliferated significantly more in response to the SPA of *R. typhi* than to the other SPAs ($P \leq 0.05$). Dose-response experiments (Fig. 2) confirm the heteroclitic nature of clones 44 and 59, as well as the highly specific response of clone 95 for the SPA of *R. typhi*. Clone 128 did not demonstrate heteroclitic responses in dose-response experiments, and this may reflect the effect of freezing and thawing since the ability of this clone to proliferate in response to specific antigen was substantially decreased between the two sets of experiments.

**Gamma interferon produced by SPA-specific CD4-positive clones.** Levels of gamma interferon produced by clones 18 and 30 in response to the SPA of *R. typhi* and by the remainder of the CD4-positive clones in response to all four SPA antigens were measured by using either a biological assay (clones 18 and 30) or an RIA. As a control, gamma interferon levels produced by all the CD4-positive clones were measured in response to medium alone. All 18 clones (Fig. 1C and D) appeared to have a minimal gamma interferon response to the SPA of *R. canada* compared with the high levels of gamma interferon generated in response to one or more of the SPAs derived from the other typhus group rickettsiae.

## DISCUSSION

The present study further establishes the role of the SPA as an important antigen for humans which is capable of stimulating a heterogeneous population of CD4-positive T cells, all of which produce gamma interferon in response to specific antigen. Previous work performed by our group has demonstrated that PBMC from all typhus-immune individuals tested (who have a diverse range of human leukocyte antigen specificities) demonstrate significant proliferative responses in the presence of SPA (3).

B cells obtained from these same immune individuals are capable of producing anti-SPA antibodies in vitro upon specific stimulation (15). However, the concentration of antigen used in the present studies is in a range which would suppress the in vitro generation of human T helper cells which provide help to these antibody-producing B cells. This would suggest that the gamma interferon-producing, CD4-positive lymphocytes identified in the present study are distinct from those which provide B-cell help. This is consistent with T helper clones described in murine systems (6).

Additional evidence that the SPAs derived from different species and strains of typhus group rickettsiae represent a heterogeneous group of proteins has been provided by examination of the cleavage products of these proteins by endopeptidases (W. M. Ching and G. A. Dasch, FASEB J. 2:A1781, 1988) and by differences in SPA electrophoretic mobility by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (8). The observation that almost all of the clones do recognize, to some extent, the SPAs from *R. typhi* and *R. prowazekii* (Breinl or Madrid E strain) is consistent with early clinical observations that infection with one of these strains leads to immunity to the other (18). Differences between the SPAs of the Breinl and Madrid E strains of *R. prowazekii* apparently do not alter the ability of the Madrid E strain to confer protective immunity but might account for the alteration in virulence observed in this strain (10).

We suggest, given the results of the present study and of previous studies in animal models (2), that the SPAs of

typhus group rickettsiae form the basis for immunity to typhus group rickettsiae and will serve as potential subunit vaccines against typhus.

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