NOTES

Lymphokine-Activated-Killer-Mediated Lysis of Cells Infected with Typhus Group Rickettsiae Can Be Inhibited by OKT3 Monoclonal Antibody

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We recently described a population of human lymphocytes which were positive for T3 and T8 lymphocyte surface antigens and which were capable of lysing typhus group rickettsia-infected targets (5). These effectors could be generated by stimulating the peripheral blood mononuclear cells (PBMC) of immune individuals with typhus group rickettsia-derived antigen or by stimulating the PBMC of both immune and nonimmune individuals with interleukin-2 (5). Because these effectors lyse both autologous and human lymphocyte antigen-mismatched targets infected with typhus group rickettsiae, and because stimulation with interleukin-2 alone is sufficient for their generation, we have classified these effectors as lymphokine-activated killers (LAK) (9). Although the ability of LAK to lyse various tumor targets in vitro and to inhibit the growth of these tumors in vivo has been well described (7, 11), the ability of LAK to lyse targets infected with intracellular pathogens has not yet been explored.

To further understand the lytic process and to confirm the phenotypic characterization of these cytotoxic effectors, we designed a series of experiments to determine whether the LAK-induced lysis of typhus group rickettsia-infected targets could be inhibited by monoclonal antibodies directed against the T3, T4, and T8 lymphocyte surface antigens. It has been reported previously (6, 13) that monoclonal antibodies directed against the T3, T4, and T8 antigens are capable of inhibiting the lysis of appropriate targets by cytotoxic T lymphocytes (CTL), thus suggesting that each of these lymphocyte surface antigens can play a role in the lytic process. However, the involvement of these antigens in the lysis of appropriate targets by LAK has not yet been investigated. The results of the present study indicate that the T3 antigen, but not the T4 or T8 antigen, is involved in the LAK-mediated lysis of targets infected with typhus group rickettsiae.

Antigens were prepared as described previously (5). PBMC were obtained by Ficoll-Hypaque centrifugation of diluted peripheral venous blood (4) from individuals who had serologic evidence of infection with either Rickettsia typhi or Rickettsia prowazekii, as determined by an enzyme-linked immunosorbent assay (10), with or without a history of clinical rickettsial disease. PBMC were stimulated in vitro for 7 days with typhus group rickettsia-derived antigen as previously described (5).

Both phytohemagglutinin (PHA)-induced blast cells and lymphoblastoid cell lines (LCL) were used as targets in the cytotoxicity assays and were generated as described previously (5). A portion of these targets was infected with either R. typhi or R. prowazekii at a multiplicity of infection (MOI) of 4 (5). The remainder of the target cells were left uninfected but treated in an identical manner.

Cytotoxicity was determined in a 4- or 6-h 51Cr-release assay (5), and each effector/target ratio was assayed in three replicates. Percent specific cytotoxicity was calculated as follows: % specific lysis = (experimental release − spontaneous release)/(maximum release − spontaneous release) × 100. The ratio of spontaneous release to maximum release was always less than 0.30. The data was analyzed by using the Student t test, and statistical significance was assigned at P < 0.05.

Affinity-purified OKT3 and OKT4 antibodies and OKT8 antibody as mouse ascites were kindly provided by William E. Biddison, National Institutes of Health, Bethesda, Md. Concentrations of antibodies used in the inhibition studies described below were greater than that necessary to saturate the appropriate binding sites, as determined by the binding of the respective fluorescein isothiocyanate-labeled antibodies to various populations of PBMC using the FACS II (2). OKT3 or OKT4 antibody (10 μg) was incubated with 6 × 10⁶ antigen-stimulated PBMC in 0.6 ml of medium for 30 min before addition of the infected targets. Similarly, 100 μl of OKT8 ascites fluid was added to 6 × 10⁶ antigen-stimulated PBMC in 0.5 ml of medium (final dilution of 1:6) for 30 min before addition of the infected targets.

Preincubation of effectors with OKT3 antibody inhibited the observed lysis of typhus group rickettsia-infected PHA blast cells in two experiments by 87.3 and 86%, respectively (Table 1). Similarly, in two additional experiments (Table 2), preincubation with OKT3 antibody inhibited the lysis of the typhus group rickettsia-infected PHA blast cells by 54 and 86%, respectively, whereas preincubation with either OKT4

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or OKT8 antibody resulted in no significant inhibition of lysis.

Because the antibodies were capable of binding to both effectors and targets in the experiments described above, it was not clear whether the inhibition was due to blocking at the level of the effectors or the target. By using LCL targets to which the antibodies did not bind (data not shown; [15]), we determined that the observed inhibition occurred at the level of the effector (Table 3). Again, preincubation with OKT3 antibody inhibited the lysis of the infected LCL by 66.8%, whereas preincubation with OKT8 antibody resulted in no inhibition.

The T3, T4, and T8 lymphocyte surface antigens are involved in the lysis of appropriate targets by CTL (13). The T3 antigen, which consists of four structurally distinct glycoproteins (3), has been shown to play a role in human-T-cell-mediated cytotoxicity at a step subsequent to killer-target recognition and adhesion (15). We have recently described the LAK-mediated lysis of human PHA blast cells or LCL which had been infected with typhus group rickettsiae (5). Although the phenotype of LAK has been well characterized (5, 8) as being T3- and T8-positive, the role of these surface antigens in the lysis of susceptible targets has not been reported.

The results of the present study clearly demonstrate that the T3 antigen, but not the portions of the T4 or T8 antigen recognized by OKT4 and OKT8 antibodies, respectively, is involved in LAK-mediated lysis of targets infected with typhus group rickettsiae. This is in contrast to studies involving murine LAK, which suggest that LAK-mediated lysis can be inhibited by the addition of anti-Lyt-2 monoclonal antibody to cytotoxicity assays (14). However, since others have demonstrated both that different monoclonal antibodies directed against T8 differ in their abilities to block CTL activity directed against allogenic targets (M. L. Stern and C. F. Ware, Abstr. Annu. Meet. Am. Soc. Microbiol. 1985, E77, p. 87), and that there is considerable variability in the ability of OKT8 antibody to inhibit CTL-mediated lysis (6, 12, 13), the present observations do not completely rule out the involvement of the T8 antigen in LAK-mediated lysis. Since the OKT8 antibody preparation used in the present study has been shown to block the lysis of allogenic targets by CTL directed against SB antigens (1), the inability of the OKT8 antibody to block the lysis of rickettsia-infected targets in the present study is unlikely to be due to the use of an ineffectual antibody.

It has been reported previously that LAK precursors, unlike mature LAK, do not bear the T3 or T8 surface antigen (8). Consequently, the acquisition of the T3 antigen during the maturation of LAK may be a critical step in the development of a fully functional cytotoxic effector.

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