AD-A202 172

REPORT DOCUMENTATION PAGE

DTIC FILE COPY

Field	Content
1b. RESTRICTIVE MARKINGS	
2a. SECURITY CLASSIFICATION AUTHORITY	
2b. DECLASSIFICATION / DOWNGRADING SCHEDULE	
3. DISTRIBUTION / AVAILABILITY OF REPORT	Approved for public release; distribution is unlimited
4. PERFORMING ORGANIZATION REPORT NUMBER(S)	NMRI 88-63
5. MONITORING ORGANIZATION REPORT NUMBER(S)	
6a. NAME OF PERFORMING ORGANIZATION	Naval Medical Research
6b. OFFICE SYMBOL (If applicable)	
7a. NAME OF MONITORING ORGANIZATION	Naval Medical Command
6c. ADDRESS (City, State, and ZIP Code)	Bethesda, Maryland 20814-5055
7b. ADDRESS (City, State, and ZIP Code)	Department of the Navy Washington, D.C. 20372-5120
8a. NAME OF FUNDING/SPONSORING ORGANIZATION	Naval Medical Research and Development Command
8b. OFFICE SYMBOL (If applicable)	
9. PROCUREMENT INSTRUMENT IDENTIFICATION NUMBER	
8c. ADDRESS (City, State, and ZIP Code)	Bethesda, Maryland 20814-5055

10. SOURCE OF FUNDING NUMBERS

PROGRAM ELEMENT NO.	PROJECT NO.	TASK NO.	WORK UNIT ACCESSION NO
62770A	3M162770A870	AQ-120	DA313964

11. TITLE (Include Security Classification)
Recognition of typhus group rickettsia-infected targets by human lymphokine-activated killer cells

12. PERSONAL AUTHOR(S) Carl M, Ching W, Dasch GA

13a. TYPE OF REPORT	13b. TIME COVERED	14. DATE OF REPORT (Year, Month, Day)	15. PAGE COUNT
Journal article	FROM ______ TO ______	1988	4

16. SUPPLEMENTARY NOTATION
Reprinted from: Infection and Immunity Sept 1988 Vol. 56(9) pp. 2526-2529

17. COSATI CODES

FIELD	GROUP	SUB-GROUP

18. SUBJECT TERMS (Continue on reverse if necessary and identify by block number)
Lymphokine activated killer; Rickettsia; Typhus; Cytoxixity

19. ABSTRACT (Continue on reverse if necessary and identify by block number)

DTIC ELECTE DEC 3 0 1988 S D E

20. DISTRIBUTION/AVAILABILITY OF ABSTRACT	21. ABSTRACT SECURITY CLASSIFICATION
☒ UNCLASSIFIED/UNLIMITED ☐ SAME AS RPT. ☐ DTIC USERS	Unclassified

22a. NAME OF RESPONSIBLE INDIVIDUAL	22b. TELEPHONE (Include Area Code)	22c. OFFICE SYMBOL
Phyllis Blum, Information Services Division	202-295-2188	ISD/ADMIN/NMRI

DD FORM 1473, 84 MAR — 83 APR edition may be used until exhausted. All other editions are obsolete.

SECURITY CLASSIFICATION OF THIS PAGE
UNCLASSIFIED

88 12 29 061

INFECTION AND IMMUNITY, Sept. 1988, p. 2526–2529 Vol. 56, No. 9
0019-9567/88/092526-04$02.00/0
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Recognition of Typhus Group Rickettsia-Infected Targets by Human Lymphokine-Activated Killer Cells

MITCHELL CARL,* WEI-MEI CHING, AND GREGORY A. DASCH

Rickettsial Diseases Division, Infectious Diseases Department, Naval Medical Research Institute, Bethesda, Maryland 20814-5055

Received 12 August 1987/Accepted 21 May 1988

Lymphokine-activated killer cells caused significant lysis of target cells infected with typhus group rickettsiae (TGR) but not cells infected with *Rickettsia tsutsugamushi*. Monoclonal and polyclonal antibodies against TGR or TGR-infected cells failed to bind specifically to infected targets, as determined by flow cytometry or by immunoprecipitation of radiolabeled cell surface proteins. Major unaltered antigenic determinants of TGR therefore cannot be detected on the surface of infected targets.

We have demonstrated previously that phytohemagglutinin (PHA)-induced blasts and lymphoblastoid cell lines (LCL) infected with typhus group rickettsiae (TGR) are susceptible to lysis by a human cytotoxic effector which is T3 and T8 positive (3), and both autologous and human leukocyte antigen (HLA)-mismatched infected targets are susceptible to such lysis. Since this cytotoxic effector was also generated by stimulation of peripheral blood mononuclear cells (PBMC) with interleukin-2 (IL-2) in the absence of specific antigen, its properties resemble those of lymphokine-activated killer cells (LAK) (9). More recently, we have cloned classical HLA-restricted human cytotoxic T cells (CTL) which specifically recognize TGR-infected cells but not *Rickettsia tsutsugamushi*-infected cells (4). The present studies were designed to determine whether the ability of TGR to render a cell susceptible to LAK lysis is shared with the antigenically distinct rickettsial species *R. tsutsugamushi* and whether infection with either of these agents is associated with specific alterations in the host cell surface membrane which might serve as a recognition structure for either antigen-specific CTL or LAK.

We compared the ability of LAK to lyse targets infected either with TGR or with an antigenically distinct rickettsial species, *R. tsutsugamushi*. PBMC were obtained from two individuals who had both clinical and serologic (11) evidence of previous infection with *R. typhi* and from two nonimmune individuals. PBMC were obtained by Ficoll-Hypaque centrifugation of diluted venous blood as described previously (2). PBMC from immune donors were stimulated as described previously (3) with whole-cell French pressure cell extracts derived from *R. typhi* and *R. prowazekii* (6), and PBMC from nonimmune donors were stimulated with recombinant human IL-2 (rIL-2), which was generously donated by Cetus Corporation (Emeryville, Calif.).

LCL generated from each of the donors by infecting B cells with Epstein-Barr virus (14) served as targets. They were infected with either *R. typhi* (Wilmington strain), *R. prowazekii* (Breinl strain), or *R. tsutsugamushi* (Gilliam strain) at a multiplicity of infection (MOI) of 4 (5, 17, 20). In order to confirm that targets were appropriately infected, cells infected with *R. prowazekii* or *R. typhi* were examined by the Gimenez procedure (20) and cells infected with *R. tsutsugamushi* were stained with Giemsa (5). ^{51}Cr-labeled targets and effectors were then incubated at different effector-target ratios for 4 to 6 h, and cytotoxicity was determined as described previously (3). The standard error of the mean is given in all cases. The ratio of spontaneous release to maximum release was always less than 0.30.

A significantly higher percentage of LCL targets infected with TGR were susceptible to LAK-mediated lysis than were the *R. tsutsugamushi*-infected target ($P \leq 0.01$) and the uninfected target ($P \leq 0.02$) (Tables 1 and 2). Since both LAK and human CTL clones (3, 4) are capable of lysing cells infected with TGR, and since both CTL (8) and LAK (10) described in other systems recognize target cell surface structures, we tried to identify specific cell surface modifications which might serve as recognition structures of antigen-specific CTL or LAK. Supporting the idea that intracellular bacteria can elicit host cell surface alterations are recent reports that mouse L cells infected with *R. typhi* appear to preferentially bind TGR-specific antibodies (13) and that cells infected with *Chlamydia trachomatis* express lipopolysaccharide (LPS) antigen on the cell surface (18).

We therefore examined infected and uninfected target cells for the presence of rickettsia-specific cell surface antigens or cell-specific modifications by examining the binding of various antibodies to infected and uninfected targets by means of electronic cell sorting with the FACS II fluorescence-activated cell sorter (1). Antibodies used in these studies included polyclonal rabbit sera prepared against whole-cell antigen preparations of *R. prowazekii* (Breinl strain) and *R. tsutsugamushi* (Gilliam strain) (5, 20), F(ab')$_2$ fragments of high-titered immunoglobulin G antibody directed against *R. prowazekii* (Breinl strain; prepared by Jackson Immunoresearch Inc., Avondale, Pa.), and mouse monoclonal antibodies directed against multiple epitopes of the 120-kilodalton (kDa) surface protein antigen, the 60-kDa protein, and LPS derived from TGR (G. A. Dasch, M. Klingseis, W. G. Sewell, and J. Kalimon, Abstr. Annu. Meet. Am. Soc. Microbiol. 1982, abstr. no. E36, p. 152). Rabbit antibodies used in these experiments recognize major rickettsial protein antigens of 17, 60, 80, 110, 120, and 180 kDa as well as LPS. Antibodies directed against rabbit and mouse immunoglobulins were obtained from Tago, Inc., Burlingame, Calif., and monoclonal antibody directed against the HLA-DR antigen (anti-HLA-DR) was obtained from Becton Dickinson, Inc., Mountain View, Calif. We chose to use *R. prowazekii*-infected targets in this series of experiments because LCL targets infected with *R. prowazekii* are also susceptible to LAK-mediated lysis (3) and

* Corresponding author.

TABLE 1. Lysis of *R. typhi*- or *R. tsutsugamushi*-infected targets by *R. typhi* antigen-stimulated PBMC from typhus-immune donors

Individual and expt no.	Target	% Specific lysis at E:T[a] ratio:		
		50:1	12:1	3:1
A				
Expt 1	*R. typhi* infected	48.1 ± 2.7	36.3 ± 0.7	22.3 ± 1.4
	R. tsutsugamushi infected	9.2 ± 0.9	6.9 ± 0.7	6.1 ± 0.3
	Uninfected	12.7 ± 0.6	8.7 ± 0.3	4.6 ± 0.7
Expt 2	*R. typhi* infected	31.4 ± 1.4	19.3 ± 0.9	11.3 ± 1.5
	R. tsutsugamushi infected	2.8 ± 0.4	2.4 ± 0.3	1.3 ± 0.5
	Uninfected	8.9 ± 1.0	7.7 ± 0	5.6 ± 0.2
B				
Expt 1	*R. typhi* infected	62.2 ± 0.6	45.8 ± 2.4	28.1 ± 1.9
	R. tsutsugamushi infected	14.4 ± 0.8	3.5 ± 9.9	7.7 ± 1.3
	Uninfected	32.0 ± 0.4	20.3 ± 0.4	10.2 ± 1.3
Expt 2	*R. typhi* infected	15.6 ± 1.0	10.3 ± 0.6	6.7 ± 0.5
	R. tsutsugamushi infected	0.3 ± 0.6	0.3 ± 0.4	0 ± 0
	Uninfected	1.7 ± 0.6	3.4 ± 0.3	0 ± 0

[a] E:T, Effector-target.

because *R. prowazekii* does not exit the cell until very late in the infection, whereas *R. typhi* spreads rapidly from one cell to another early in the infection cycle (19).

In multiple experiments, uninfected LCL targets and LCL targets infected with either *R. prowazekii* or *R. tsutsugamushi* were incubated with the antibodies described previously; however, we were unable to detect specific binding of these antibodies by electronic cell sorting with the FACS II to any of the targets or to uninfected control targets, despite extensive binding of positive control antibodies to infected and uninfected targets (data not shown). We believe that the antibodies used in these experiments have broad reactivity against all known major immunogenic protein and LPS determinants found on *R. prowazekii* and *R. tsutsugamushi* (Gilliam strain).

As an alternative method, we attempted to identify rickettsia-specific cell surface antigens by performing polyacrylamide gel electrophoresis (12) of ^{125}I-labeled uninfected, *R. prowazekii* (Breinl strain)-infected, or *R. tsutsugamushi* (Gilliam strain)-infected target cell surface proteins radiolabeled and immunoprecipitated as described previously (16). We were unable to precipitate any cell surface proteins which reacted specifically with antibody directed against *R. prowazekii* or against *R. tsutsugamushi*, although there was successful precipitation of the HLA-DR antigen complex (Fig. 1).

We also investigated the possibility that TGR induces the host cell to present some type of altered self-structure recognized by the LAK effector. A 3-month-old New Zealand White rabbit was immunized intravenously once weekly for 3 consecutive weeks with 10^7 LCL infected with *R. prowazekii* at an MOI of approximately 4. At the end of the third week, immune serum was obtained, and we studied the ability of this immune serum to bind to the uninfected and infected LCL lines used in the immunizations. After antibodies from the immune serum which cross-reacted with uninfected targets were adsorbed, we were unable to detect binding of either the immune serum or the nonimmune serum (obtained from the same rabbit prior to immunization) to either uninfected LCL or LCL infected with *R. prowazekii*. Thus, we were unable to detect any cell surface alterations induced by infection with *R. prowazekii*.

The absence of detectable antigen on the surface of infected targets susceptible to lysis by a cytotoxic effector does not appear to be a property limited to the rickettsiae. Similar problems in detection of antigens of *Theileria parva* (7) or influenza virus (15) on the cell surface of targets susceptible to lysis by CTL have been recently described (7). Yet, according to classical dogma, our recent demonstration of HLA-restricted TGR-specific human CTL would require the presence of a surface-localized specific rickettsial antigen which is associated with a host cell class II HLA antigen (4). Our failure to detect any of the major antigens of TGR on the surface of infected cells may be due to (i) the processing of one of the major immunogenic antigens by the target cell so that it is no longer recognized by any of our specific antibodies or (ii) the target cell expression of a "minor" rickettsial antigen not recognized by the antibodies used. In the case of the LAK effectors, it is possible that recognition of an infected target occurs via a non-rickettsia-specific cell

TABLE 2. Lysis of *R. typhi*- or *R. tsutsugamushi*-infected targets by rIL-2 PBMC from nonimmune donors

Individual	Target	% Specific lysis at E:T[a] ratio:		
		50:1	12:1	3:1
A	*R. typhi* infected	52.2 ± 2.9	56.3 ± 2.1	49.2 ± 1.7
	R. tsutsugamushi infected	0 ± 0	2.8 ± 0.2	1.5 ± 2.0
	Uninfected	5.2 ± 1.5	7.7 ± 1.0	1.3 ± 0.8
B	*R. typhi* infected	32.3 ± 2.0	27.2 ± 1.0	17.3 ± 2.0
	R. tsutsugamushi infected	14.1 ± 1.4	10.9 ± 2.8	5.5 ± 1.8
	Uninfected	15.4 ± 2.8	13.2 ± 1.4	9.7 ± 1.1

[a] E:T, Effector-target.

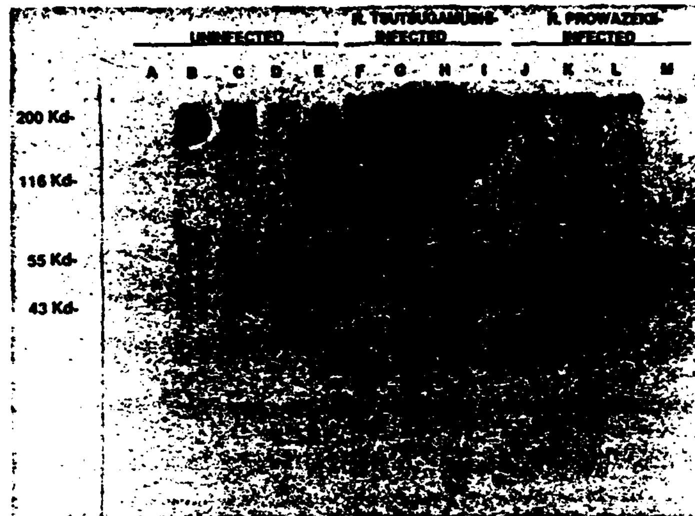

FIG. 1. LCL were either infected with *R. prowazekii* at an MOI of 4 (lanes J to M), infected with *R. tsutsugamushi* at an MOI of 4 (lanes F to I), or left uninfected (lanes A to E). After 3 days, the infected and uninfected populations were harvested, and surface proteins were labeled with ^{125}I. After solubilization of the surface membrane proteins with Nonidet P-40, iodinated proteins were incubated with polyclonal rabbit serum with high titers of antibodies directed against *R. prowazekii* (lanes A, F, and J) or *R. tsutsugamushi* (lanes C, H, and L). As a negative control, iodinated proteins were incubated with polyclonal rabbit serum obtained prior to immunization of the rabbits with *R. prowazekii* (lanes B, G, and K) or *R. tsutsugamushi* (lanes D, I, and M). As a positive control, iodinated surface proteins were incubated with monoclonal antibody directed against HLA-DR (lane E). Antigen-antibody mixtures were then incubated with protein A-Sepharose CL-4B and solubilized with sample buffer containing sodium dodecyl sulfate. The samples were then analyzed by electrophoresis on a 12% polyacrylamide gel, and an autoradiogram of the dried gel was prepared with intensifying screens. Films were exposed for 10 days at −70°C. Molecular mass (in kilodaltons) is shown to the left.

surface alteration not detectable by the rabbit antibodies generated against infected cells.

The surface alteration(s) induced by infection of targets with TGR which is recognized by cytotoxic effectors (CTL or LAK) will clearly have to be identified by methods other than those described in the present study. Accordingly, workers in our laboratory are now in the process of cloning the major antigens of TGR and attempting to express these genes in eucaryotic cells by means of appropriate vectors.

This investigation was supported by the Naval Medical Research and Development Command, Department of the Navy, Research Task No. 3M162770A870.AE.307.

We thank Richard Wistar, Barry Bloom, and Jim Murphy for helpful discussions; Doug McDonald, Walter Sewell, Richard Grays, and Sharon Brewer for technical assistance; Neil Hardegan for FACS II analysis; and Betty Jo Leckey and Morna Gonsoulin for manuscript preparation.

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