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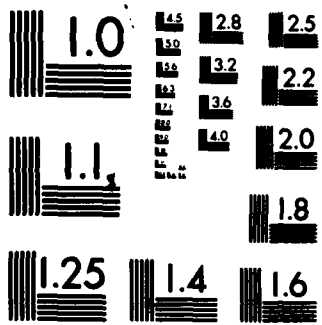
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**Characterization of Antibody-Dependent Killing
of Trypanosomes by Macrophages.**

Truncated Annual Summary Report

David L. Rosenstreich, M.D. and
Hellen C. Greenblatt, Ph.D.

March 1983

For the period 15 September 1981 - 14 January 1982

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U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
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Albert Einstein College of Medicine
Bronx, New York 10461

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<input checked="" type="checkbox"/> Macrophages will bind trypanosomes in the presence of variant-specific antibody. Monoclonal antibodies are specific for the variant against which they were raised. Ability to bind trypanosomes correlates best with the presence of a monoclonal antibody that by immuno-fluorescence binds to surface components of trypanosomes.		

Foreword

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Project Entitled: Characterization of antibody-dependent killing of trypanosomes by macrophages.

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TRUNCATED ANNUAL REPORT

Submitted December 21, 1981

The following is a summary of work accomplished on the above project entitled "Characterization of antibody-dependent killing of trypanosomes by macrophages" during the period September 15, 1981 to December 21, 1981.

1) The macrophage/trypanosome assay was successfully re-established in our laboratory. WRATat-300 (Wellcome) trypanosomes bind to resident macrophages in the presence of variant specific antibody in a dose dependent fashion. Uptake was enhanced in the presence of guinea pig complement. Anne Lazo, our technician, was trained to read slides and is now able to perform this task independently and efficiently.

2) On October 6, 1981 Dr. Greenblatt met at WRAIR with Dr. Jeenan Tseng and Klaus Esser to arrange for the necessary collaboration for the contract studies. Dr. Tseng agreed to prepare immunoglobulin fractions from rabbit antisera raised against trypanosomes. The procedures and schedule for shipment of monoclonal antibodies was discussed with Klaus Esser.

3) On November 11, 1981 we received 24 ascites fluids containing hybridoma derived anti-T. rhodesiense WRATat-1 products and the WRATat-1 p10 stabilate. Since all of these monoclonal have anti-WRATat-1 specificity it will be necessary to use this VAT in the macrophage/trypanosome assay. We have, therefore, prepared a stabilate of WRATat-1 and have obtained experience with its passage requirements. This strain, which has growth kinetics markedly different from WRATat-300 is now successfully established in the laboratory.

4) An initial series of experiments will be performed to test the antigenic homogeneity of the WRATat-1 and the antigenic specificity of the anti-WRATat-1 antiserum. Once we complete these preliminary experiments the first of our monoclonals will be ready for screening. Following this, we will proceed with the experiments outlined in the contract.

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Submitted January 28, 1982

The following is a summary of work accomplished in the above project during the initial four month period.

1) In order to test the specificity of this system, a monoclonal antibody generated against WRATat-1 and a polyclonal rat anti-WRATat-300 antiserum were utilized to mediate, in the presence of guinea pig complement, binding of WRATat-1, 186, and 300 to resident macrophages. The results were as follows:

TABLE I

<u>Serum</u>	<u>Trypanosome</u>		
	<u>WRATat-1</u>	<u>WRATat-186</u> <u>(EATRO 1886)</u>	<u>WRATat-300</u> <u>(Wellcome)</u>
1. monoclonal 2.1B7.1 (anti-WRATat-1)	+	-	-
2. anti-WRATat-300	-	-	+
3. NMS	-	-	-

2.1B7.1 was only able to mediate binding of WRATat-1, and the anti-WRATat-300 product bound WRATat-300 trypanosomes to mouse resident PEC, demonstrating the specificity of this system (Table I).

2) In November, 1981 we received 24 ascites fluids containing hybridoma derived anti-T. rhodesiense WRATat-1 antibodies. Seven of these were tested for activity in the presence of rat serum complement. Two antibodies (4.2H3.1 and 4.1C4.1) were very positive even in the absence of complement (Table II).

TABLE II

	<u>% of Macrophages Binding Trypanosomes</u> <u>(% Complement)</u>				<u>Antibody Activity By</u> <u>Immunofluorescence *</u>	
	12.5	6.25	3.13	0	<u>Cytoplasmic and</u> <u>Surface Binding</u>	<u>Surface Binding</u> <u>Only</u>
Monoclonal (final 1:20)						
2.1B7.1	35	51	13	9	+	+
4.1A12.1	15	9	ND	ND	-	-
4.1C4.1	23	55	70	73	+	+
4.1E6.1	3	3	2	3	+	-
4.1G9.1	4	10	3	0	+	-
4.2H3.1	53	60	80	36	+	+
6.7H11	68	47	43	8	+	+
Normal Mouse Sera	ND	2	0	1		

*Data provided by Mr. Klaus Esser (WRAIR)

Two antibodies (2.1B7.1 and 6.7H11) were positive but only in the presence of complement. The remaining three antibodies (4.1A12.1, 4.1E6.1 and 4.1G9.1) were negative. No antibodies were trypanocidal in the absence of macrophages. Comparison with a trypanosome binding assay suggest that functional activity correlates best with the presence of an antibody that binds to a surface, rather than a cytoplasmic component.

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