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11. Supported by PHS grants NS05368 and NS06735; NIH training grant NS05596; the Nancy Louise Tryner memorial grant 433-D-10 from the National Multiple Sclerosis Society; M.B.B. is a scholar of the Rose F. Kennedy Center for Research in Mental Retardation and Human Development.

27 July 1971; revised 24 September 1971

Antibody-Dependent Lymphoid Cell–Mediated Cytotoxicity: No Requirement for Thymus-Derived Lymphocytes

Abstract. *The capacity of lymphoid cells from nonsensitized mice to lyse antibody-coated target erythrocytes in vitro does not require the presence of thymus-derived or thymus-dependent lymphocytes. Thus, spleen cells from thymus-deprived mice and spleen cell populations from which thymus-dependent lymphocytes had been removed were fully competent to mediate destruction of antibody-coated target cells. However, prior treatment of spleen cell populations with antibody to κ chains diminished this function, suggesting a role for bone marrow–derived lymphocytes.*

Lymphoid cell–mediated cytotoxicity has been studied as a model for allograft and tumor immunity. Several models of cell-mediated destruction of target cells in vitro have been described (*1*). In one instance, lymphocytes from sensitized donors cause the lysis of target cells. In this model, thymus-derived or thymus-dependent (T) lymphocytes are required for cell destruction (*2*).

By contrast lymphoid cells from nonsensitized donors lyse antibody-coated target cells (antibody-dependent cell-mediated cytotoxicity) (*3*) or lyse target cells in the presence of phytohemagglutinin (*4*). We now report that antibody-dependent lymphoid cell–mediated cytotoxicity does not require the presence of T lymphocytes. Thus, spleen cells from nonsensitized, thymus-deprived mice and spleen cell populations from which T lymphocytes have been removed by lysis with antiserum to θ (an antigen on the surface of T lymphocytes) together with complement are fully competent to mediate this reaction. In addition, prior treatment of spleen cell populations with antibodies to immunoglobulin light chains of the κ class diminishes their capacity to lyse antibody-coated target cells.

Table 1. Capacity of spleen cells from thymus-deprived mice to mediate antibody-dependent cytotoxicity.

Cells ($\times 10^6$)	Anti-serum	Lysis (%) Exp. 1	Lysis (%) Exp. 2
		Intact	
5	+	74.1	61.2
2.5	+	57.7	
1.25	+	40.9	
5	−	7.5	7.1
		Thymus-deprived	
5	+	83.1	82.2
2.5	+	72.7	
1.25	+	57.4	
5	−	8.8	8.3
0	+	12.6	

The ability of lymphoid cells to effect antibody-dependent cell-mediated cytotoxicity was evaluated by the release of radioactivity from antibody-sensitized burro erythrocytes labeled with ^{51}Cr. A lymphoid cell suspension (1 ml; 1.25 to 5×10^6 cells) in tissue culture medium (*5*) was mixed with ^{51}Cr-labeled (*6*) burro erythrocytes (0.5 ml; 2×10^5 cells) and with heat-inactivated, guinea pig antiserum to burro erythrocytes (25 μl of a 1:2 dilution) (*7*); the final dilution was 1 : 130. Controls without added lymphocytes and others without added antiserum were always included in the experiments. Unlabeled sheep erythrocytes (3×10^7; 0.1 ml) were added to all mixtures to prevent spontaneous lysis of the target cells (*6*). The reaction mixtures were incubated in duplicate tubes at 37°C in an atmosphere of 95 percent air and 5 percent CO_2 for 24 hours, and the total radioactivity was measured. After centrifugation for 10 minutes at 1000 rev/min, the radioactivity of a measured portion of supernatant was determined. Cell lysis was expressed as the percentage of the total radioactivity released from cells into the supernatant.

Thymus-deprived mice are deficient in T lymphocytes (*8*). Therefore the effectiveness of a spleen cell population from CBA mice that had been thymectomized, lethally irradiated, and reconstituted with syngeneic bone marrow cells (*9*), was compared with that of spleen cells of normal CBA mice (Table 1). The spleen cells from thymus-deprived animals were no less capable of mediating cytotoxicity than were cells from normal mice. Indeed, they were somewhat more effective than cells from normal mice. This strongly suggests that T lymphocytes are not necessary for this type of lymphocyte-mediated cytotoxicity.

Another approach to the analysis of the relative role of T lymphocytes is to remove such cells from lymphoid cell populations of normal animals through immune cytolysis with antiserum to θ (anti-θ) (*10*). Spleen cell suspensions were prepared from 8-week-old male BALB/c mice. Lymphocytes bearing the θ antigen were removed from the population as follows: Fifty million cells were incubated with 0.2 ml of undiluted AKR antiserum to θ · C3H (*11*), in a total of 1.0 ml of tissue culture medium for 15 minutes at 4°C. The cells were layered on fetal calf serum (FCS) and centrifuged to remove unbound antiserum; the sedimented cells were incubated in 1.0 ml of medium containing a 1:8 dilution of guinea pig complement (*12*) for 30 minutes at 37°C. The cells were then washed four times by centrifugation through FCS (as described above) and enumerated. Control populations were treated in an identical fashion, except that antiserum was omitted. As a measure of the effectiveness of depletion, the number of θ-positive lymphocytes remaining after the above procedures was estimated as follows. Portions of the lymphoid cells were labeled with ^{51}Cr

Table 2. Effect of treatment of mouse spleen cells with anti-θ and complement (C) on their capacity to mediate antibody-dependent cytotoxicity.

Spleen cells: Prior treatment	Spleen cells: θ-Positive remaining (%)	Spleen cells: No. ($\times 10^6$)	Lysis (%)
	Experiment 1		
Medium + C	20	5	76.3
		2.5	61.2
		1.25	41.8
Anti-θ + C	0	5	74.9
		2.5	57.9
		1.25	41.7
	Experiment 2		
Medium + C	30.9	5	41.6
Anti-θ + C	0	5	40.1

(*13*) and samples (5×10^4 cells) were incubated with equal volumes of a 1:10 dilution of anti-θ and complement; ^{51}Cr release from these cells was then measured, and the percentage of cells killed was calculated (*14*).

The relative antibody-dependent cell-mediated cytotoxicity of control spleen cell populations and of populations depleted of θ-positive cells is shown in Table 2 together with the percentage of remaining θ-positive cells. Prior treatment with anti-θ and complement (Table 2, experiments 1 and 2) resulted in elimination of all θ-positive cells without lessening the ability of the population to cause lysis of target erythrocytes. These results indicate that T lymphocytes are not required in this system.

The other major class of lymphocytes in peripheral populations which has been thus far described is the "bone-marrow derived" or B lymphocyte. This cell lacks the θ antigen but has easily detectable amounts of immunoglobulin (Ig) on its surface (*15*). This cell may therefore be removed from spleen cell populations by cytolysis with antibody to κ chains (*16*) together with complement. Such treatment of spleen cell populations diminishes their capacity to participate in antibody-dependent cell-mediated cytotoxicity, to an extent commensurate with the destruction of Ig-bearing cells, as measured by residual cells capable of being lysed by antibody to κ chains and complement (Table 3, experiments 1 and 2). Nonetheless, as is discussed below, this experiment does not definitively establish the participation of the B lymphocyte because pretreatment of spleen cell populations with antibody to κ chains without complement, while causing no lysis of B cells, is quite effective in reducing the cytotoxicity of the treated spleen cell populations (Table 3, experiment 3).

Thus in contrast to their established role in target cell destruction by lymphocytes from sensitized animals, T lymphocytes are not required for antibody-dependent cell-mediated cytotoxicity in vitro. The report of MacLennan and Harding (*17*) that spleen cells from rats which had been subjected to thoracic duct drainage were competent to cause antibody-coated target cell destruction is consistent with our observations.

Which cell type is primarily responsible for destruction of antibody-coated target cells has not yet been established. Although the finding that prior treatment of spleen cells with antibody to κ chains and complement has a marked effect on their activity suggests a role for B lymphocytes, this observation must be interpreted with caution in view of the capacity of antibody to κ chains without complement to have a similar effect. In other systems, immunologic effects attributed to cells other than B lymphocytes have been inhibited by treatment of lymphoid cell populations with antiserums to Ig in the absence of complement (*18*). Further experiments will be required to establish whether the B lymphocytes as a class or a subpopulation of B lymphocytes are indeed the effector cells. Alternatively, it is possible that an entirely different class of cells is responsible for all or some of this effect. In this context, it should be pointed out that polymorphonuclear leukocytes (PMN's) can also destroy antibody-coated target cells (*19*). It is very unlikely, however, that the PMN's are the spleen cell type mainly responsible for this effect in our experiments, as the frequencies of PMN's in our spleen cell suspensions (< 2 percent) could account for no more than 20 percent of the cytotoxicity observed (*20*). Furthermore, cooperative interactions between different cell types present in spleen cell populations may be involved in antibody-dependent cell-mediated cytotoxicity.

The mechanism of this antibody-dependent cell-mediated cytotoxicity remains unclear. It seems reasonable to postulate, however, that an initial interaction between effector cell and target cell occurs, perhaps through the receptor on B lymphocytes for antigen-antibody complexes which Miller *et al.* (*21*) have described or through the receptor for the C3 component of complement on B lymphocytes which Bianco *et al.* have reported (*22*). A subsequent synthesis by the effector cell of substances capable of inducing cell damage would then be responsible for the local destruction of the target cell. Among such cytotoxic substances may be complement components or members of the recently described alternate pathway leading to activation of C3 (*23*).

Reports have appeared indicating that under certain experimental circumstances the passive administration of large amounts of antibody directed against target organs or allografts may cause destruction of these tissues; furthermore, in these instances histologic examination often demonstrates mononuclear cells and lymphocytes at the site of the destructive processes. Damage of the target tissue by host lymphoid cells cooperating with passively administered antibody may explain some of these results. Antibody-dependent lymphocyte-mediated cytotoxicity may be an important pathway for damage to tissues observed in certain naturally occurring pathological processes (*24*).

Table 3. Effect of treatment of mouse spleen cells with antibody to κ chains on their capacity to mediate antibody-dependent cytotoxicity. The percentage of diminution (D) of the cytotoxic effect was determined by plotting in each experiment the percentage of lysis against the number of lymphoid cells from control populations, and from such curves estimating the number of control cells (N) required to yield the same amount of lysis as 5×10^6 experimental cells. Thus $D = 100[1 - (N/5 \times 10^6]$. C is complement.

Cell treatment	κ-Positive cells		Number of cells ($\times 10^6$)	Lysis (%)	Diminution of cytotoxic effect (%)
	Cells remaining (%)	Diminution (%)			
		Experiment 1			
Medium + C*	40		5	74.6	
			2.5	57.9	
			1.25	38.9	
Anti-κ + C†	17	58	5	49.4	62
		Experiment 2			
Medium + C*	36		5	86.0	
			2.5	74.0	
			1.25	55.0	
Anti-κ + C†	14	61	5	54.3	76
		Experiment 3			
Medium*	43		5	50.0	
			2.5	32.3	
			1.25	21.9	
Anti-κ†	38	12	5	24.6	67
			2.5	18.2	
			1.25	13.4	
Anti-κ(1 : 10)†	50	−16	5	31.0	53
			2.5	20.9	
			1.25	14.7	

* Control series. † Experimental series.

JOHN A. VAN BOXEL, JOHN D. STOBO
WILLIAM E. PAUL, IRA GREEN
Laboratory of Immunology, National Institute of Allergy and Infectious Diseases, Bethesda, Maryland 20014

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12. Guinea pig complement (Baltimore Biological Laboratories) was screened for naturally occurring cytotoxicity, which, if present, was removed by absorption with either thymus or spleen cells at 4°C for 1 hour.
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14. ^{51}Cr-labeled lymphocytes (5×10^4 in 50 μl) were incubated in duplicate with an equal volume of a 1:10 dilution of antiserum for 15 minutes at 4°C. Complement (50 μl of a 1:4 dilution) was added, and the mixture was incubated for 30 minutes at 37°C. After centrifugation (1500 rev/min for 10 minutes), the radioactivity of 50 μl of supernatant was measured. Total releasable radioactivity was obtained by freeze-thawing the controls three times. Other controls contained cells and medium only or cells plus complement and medium or normal mouse serum (NMS). These controls gave 10 percent or less of chromium release. The percentage of lysis is equal to $(P - R)/(Q - R)$ where P is the radioactivity released by antiserums and complement; R is the radioactivity released by NMS and complement; and Q is the radioactivity released by freeze thawing.
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16 August 1971

Gonadotropin Secretion during Sleep in Normal Adult Men

Abstract. *Release of luteinizing hormone and follicle stimulating hormone during sleep in young adult men occurred in unrelated, random, arrhythmic peaks, with no consistency from night to night in the same subject. Release of luteinizing hormone was modestly but significantly larger (14 perecnt) during rapid-eye-movement sleep than it was in non-REM sleep, but release of follicle stimulating hormone was not clearly related to stages of sleep.*

Increased secretion of adrenocorticotropic hormone (ACTH) during sleep, as reflected by cortisol levels, occurs in the early morning hours at a time when rapid-eye-movement (REM) sleep is maximal (*1*). Increased secretion of growth hormone, on the other hand, occurs primarily in the first few hours after sleep onset and is closely related to slow-wave (stages 3–4) sleep (*2*). Whereas release of ACTH can be dissociated from REM sleep by acute sleep-wake reversal (*3*), release of growth hormone follows intimately the time of onset of slow-wave sleep (*4*) and is not alterable by manipulations which affect growth hormone during waking hours (*5*). The possibility that related neural mechanisms control both sleep patterns and the secretion of some anterior pituitary hormones is enhanced by evidence that biogenic amines have neurotransmitter roles both in sleep (*6*) and in the secretion by the hypothalamus of pituitary hormone releasing and inhibiting factors (*7*). Investigation of other pituitary hormones during sleep is thus warranted.

We now report a study of luteinizing hormone (LH; ICSH) and follicle stimulating hormone (FSH) release during sleep in normal young men. Patterns of release of these two hormones in normal men have not been extensively investigated. Concentrations of both hormones, measured every 2 hours for 1 day, in serums of ten subjects were quite stable (*8*); other studies, however, showed a circadian rhythm of serum FSH in men, with highest values occurring in the early morning hours (*9*). Some periodicity of plasma LH was observed during sleep in three men, although there was no obvious relation to sleep stages (*10*). Peaks of plasma testosterone, on the other hand, were found in conjunction with episodes of REM sleep in five male subjects (*11*).

We studied 16 healthy male volunteers, ages 21 to 30, on no drugs or medication, for four consective nights in the sleep laboratory. Men were chosen in order to obviate the cyclic influence of the female hypothalamus on gonadotropins. They were allowed normal meals and activity during the day. Continuous electrophysiologic (electroencephalographic, electrooculographic, and electromyographic) recordings were made each night during the hours of sleep (11 p.m. to 7 a.m.). On the first night, only electrophysiologic recordings were made; on the second night an antecubital venous catheter was placed. but no blood was taken; on the third and fourth nights blood was sampled from an adjoining room through a 10-foot tube connected to the catheter (*12*). The first eight subjects were sampled every 30 minutes from 11 p.m. to 7 a.m.; the second eight subjects were sampled every 10 minutes between 11 p.m. and 1 a.m. and between 5 and 6 a.m. Total blood withdrawal was kept below 200 ml each night. The plasma was immediately separated and frozen until radioimmunoassay for the hormones could be done (*13*). All samples were run in duplicate, and all samples from each subject were run in the same assay. Mean intra-assay variability was 5 percent; mean inter-assay variability was 20 percent.

Each thirty-second epoch of EEG recording was scored as awake; stage 1,2,3,4; or REM by established criteria