furthermore, there was no change in the affinity of dopamine for the receptor regulating adenylate cyclase activity in these homogenates. Examination by the Falk-Hillarp technique of both substantia nigra from an additional rat which received a comparable injection of 6-hydroxydopamine demonstrated that the experimental treatment destroyed the dopaminergic nigro-neostriatal neurons on the injected side of the brain. These data demonstrate that the dopamine-sensitive adenylate cyclase in the zona reticulata of the substantia nigra can be separated from the dopaminergic nigro-neostriatal neurons and, therefore, suggest that this enzyme activity is not associated with these cells.

The presence in the substantia nigra of mechanisms (*13*) for both the uptake of dopamine and the potassium-stimulated, calcium-dependent release of dopamine, as well as the presence of dopamine receptors on both the dopaminergic neurons (*8*) and other anatomically undefined cells (our results reported here) raises the possibility that within this region of the brain dopamine may have a physiological role (or roles) similar to the roles of dopamine in the striatum, where the uptake, storage, and release of dopamine have been demonstrated and where both pre- and postsynaptic receptors for dopamine occur (*7, 13, 14*). Our results suggest that the substantia nigra might represent another site (in addition to the neostriatum) where either antipsychotic drugs or drugs used to treat Parkinson's disease could affect the regulation by dopamine of physiological activity in the extrapyramidal system (*15*).

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10. Samples of the tissue were isolated with a 300-μm (inside diameter) needle from frozen sections (300 μm) of rat brain; the zona compacta, zona reticulata, and pars lateralis of the substantia nigra are the subdivisions of the substantia nigra identified as regions 44, 45, and 46, respectively, by M. Palkovits, M. Brownstein and J. M. Saavedra [*Brain Res.* **80**, 237 (1974)]. Tissue was homogenized in a solution of 2 m*M* tris(hydroxymethyl)aminomethane maleate (tris-maleate) (*p*H 7.4) and 2 m*M* EGTA ([ethylenebis (oxyethylenenitrilo)]tetraacetate). Adenylate cyclase activity in homogenates was assayed in a mixture (final volume, 0.05 ml) containing (mmole/liter): tris-maleate, *p*H 7.4, 80; theophylline, 10; $MgSO_4$, 6.0; EGTA, 0.8; and adenosine triphosphate (ATP), 1.5; 0.01 ml of tissue homogenate containing approximately 10 μg of protein; the test compounds were added at the concentrations indicated. The assay of enzyme activity was initiated with the addition of ATP; all samples were incubated at 30°C for 5 minutes in a shaken water bath. The incubations were terminated by placing the tubes in a boiling water bath for 2 minutes. Under these assay conditions, the enzyme activity in all regions studied was proportionate to both the duration of the incubation at 30°C and the concentration of tissue homogenate. All salts were of reagent grade. Catecholamines were obtained from Sigma, Calbiochem, or Aldrich Chemical Company; chlorpromazine was supplied by Smith Kline & French. The cyclic AMP content of the entire sample was measured by the method of B. L. Brown, J. D. M. Albano, R. P. Ekins, A. M. Sgherzi, and W. Tampion [*Biochem. J.* **212**, 561 (1971)] with the use of 4 pmole (30 c/mmole) of cyclic AMP. Protein was determined by the method of O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall [*J. Biol. Chem.* **193**, 265 (1951)].
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Binding of C-Reactive Protein to Antigen-Induced but Not Mitogen-Induced T Lymphoblasts

Abstract. *The C-reactive protein (CRP), an acute phase reactant which binds selectively to T (thymus-derived) lymphocytes, was found to bind to lymphoblasts formed upon stimulation with antigens but not with mitogens. Binding of CRP thus serves as a marker for antigen-reactive (-reacted) as opposed to mitogen-reactive (-reacted) T cells, suggesting that these represent separate subpopulations, and supports the developing concept that CRP plays an important role in the regulation of responses critical to inflammation, host defense, and tissue repair.*

C-reactive protein (CRP) is a trace constituent of serum that was originally defined by its calcium-dependent precipitation with the C polysaccharide (CPS) of the pneumococcus (*1*). Its concentration in the blood, which increases during the acute phase of febrile illnesses and a variety of tissue-destructive or inflammatory processes, has long served as a clinical indicator of these states (*2*). It has been reported that CRP binds selectively to 30 to 40 percent of human peripheral blood lymphocytes, and that the lymphocytes to which the CRP binds are primarily T cells (*3*). This binding is associated with inhibition of the formation of T cell rosettes with sheep erythrocytes, the mixed lymphocyte reaction (MLR), and the generation of cytotoxic lymphocytes (*3, 4*). By contrast, proliferative responses to the T cell mitogens phytohemagglutinin (PHA) and concanavalin A (Con A) are unaltered (*3, 4*). Further, CRP by itself does not induce lymphocyte blastogenesis, is not cytotoxic for lymphocytes in culture, and does not bind to lymphocytes with complement receptors or surface immunoglobulin (*3*); it reacts with theta-bearing cells derived from mouse spleen but not from mouse thymus (*4*). Taken together, these findings suggested that CRP binds to and alters the function of a certain fraction, but not all, of T lymphocytes.

It seemed possible that the T cells binding CRP were cells at a particular stage in their reproductive cycle, cells that had been injured, or cells of a T cell subpopulation. To examine these possibilities, we studied the binding of CRP to human lymphoblasts obtained by stimulation with PHA, Con A, allogeneic cells, and purified protein derivative (PPD) of tuberculin.

For these experiments, the CRP substrate CPS was obtained as described (*5*) from a capsulated pneumococcal variant (Cs), lyophilized, and stored until use. Purified human CRP was obtained by affinity chromatography of ascites or pleural fluids on CPS covalently bound to Bio-Gel according to a method previously described (*6*); the CRP was then dialyzed against saline, sterilized with a Millipore filter, stored at 4°C, and used within 30 days. Lymphocytes were obtained by centrifugation of heparinized blood from normal human donors on Ficoll-Hypaque; they were washed three times in RPMI 1640 medium containing Penn-Strep (50 μg/ml) and 10 percent heat-inactivated fetal calf serum (International Scientific Industries), and resuspended to the appropriate concentration.

The PPD-sensitized cells were obtained from donors showing delayed cutaneous reactivity to 5 tuberculin units of PPD. All cells (10^5 cells per well) were cultured in microplates, with 2 μl of PHA (PHA-P, Difco Laboratories) per well, 5 μg of Con A (Nutritional Biochemical) per well, or 25 μg of PPD (Connaught Medical Research) per milliliter. Unidirectional MLR's consisted of equal numbers of lymphocytes and allogeneic lymphocytes inactivated by mitomycin C (Calbiochem). Both stimulated and unstimulated cells were harvested as follows: on day 5 in experiments with PHA and Con A, day 6 with the MLR, and day 7 with PPD.

The cells were washed three times in medium with fetal calf serum, incubated with purified CRP (50 μg/ml) for 18 hours at 37°C in an atmosphere of 5 percent CO_2, again washed three times with fetal calf serum medium, resuspended in 0.1 ml of PSB-BSA (0.01*M* phosphate-buffered saline, *p*H 7.2; 1 percent bovine serum albumin), and reacted with fluorescein-conjugated rabbit antiserum to human CRP (Behring Diagnostics) for 30 minutes on ice. After three washings with PBS-BSA, the cells were examined (Leitz Orthoplan Photomicroscope) by incident light fluorescence with fluorescein isothiocyanate and BG38 filters at a magnification of ×400. Lymphoblasts were considered to be those cells measuring 12 to 18 μm in diameter; cells measuring 5 to 8 μm in diameter were considered to be nonlymphoblasts, while cells of intermediate size were not counted (*7*). The percentage of cells binding CRP was determined by relating the number of cells with fluorescence to the total number of cells seen on dark-field examination. Routinely, 200 cells in each category were counted, and all experiments were performed in triplicate.

We first sought to determine whether lymphoblasts as well as resting lymphocytes were capable of binding CRP by examining all the cells from cultures stimulated with T cell mitogens. The percentages of blast cells staining for CRP after overnight incubation with CRP were only 5.2 percent in cultures stimulated with PHA and 4.1 percent in cultures stimulated with Con A (Table 1); these results were similar to those obtained by background staining. By contrast, when the blast cells from MLR- and PPD-stimulated cultures were examined, much higher proportions (22.5 percent and 26.8 percent, respectively) were found to be capable of binding CRP (Table 1). The lymphoblasts staining with CRP, like the resting cells staining with CRP in previous studies (*3*), showed patchy surface fluorescence which did not result in capping even after prolonged incubations at 37°C (Fig. 1). The ratios of the percentages of blast to nonblast cells staining with CRP were 0.11 on stimulation with PHA and 0.10 on stimulation with Con A; the ratios were 0.55 in the MLR and 0.66 on stimulation with PPD. These differences were statistically significant with a P value of $< .005$ (Student's t-test). The differences in the percentages of blasts binding CRP between the two mitogen-stimulated cultures or between MLR- and PPD-induced reactions were not significant ($P > .01$). The percentages of CRP-staining nonblast cells (40 to 46 percent) were similar in each of the four stimulated cultures and were significantly ($P < .01$) greater than the percentages (36 to 38 percent) of unstimulated cells staining with CRP.

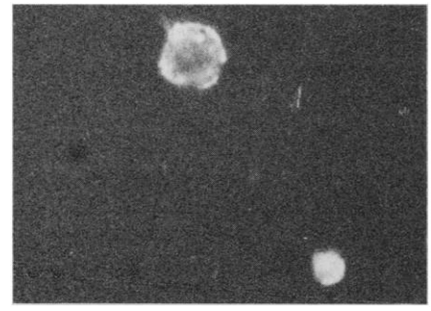

Fig. 1. Typical fluorescent staining of a lymphoblast and small lymphocyte from cells in a mixed lymphocyte reaction (MLR) which have been incubated with purified CRP (50 μg/ml) and reacted with fluorescein isothiocyanate–conjugated rabbit antiserum to human CRP. The diameter of the lymphoblast is 15 μm and the small lymphocyte 6 μm (×625).

The specificity of the fluorescence was established by incubating cells with the CRP substrate, CPS (5 or 10 μg/ml), or with unconjugated rabbit antiserum to human CRP, followed by incubation with the fluorescein-conjugated antiserum to CRP; no fluorescent staining occurred under any of these conditions. When PHA was added to nonstimulated lymphocytes simultaneously with the addition of CRP, a normal percentage (30 to 40 percent) of CRP-binding cells was obtained, indicating that this mitogen did not inhibit binding of CRP. Cell viability was similar (76 to 94 percent) in all cultures.

These experiments show that CRP reacts preferentially with antigen-induced, as opposed to mitogen-induced, lymphoblasts. They support previous observations that CRP inhibits blastogenesis induced by allogeneic cells but not by mitogens in both human and murine systems (*3, 4*) and binds with theta-bearing cells from mouse spleen (which show intense proliferative responses to allogeneic cells) but not with theta-positive cells from mouse thymus (*4*) (which do not) (*8*). Additional support for the selective binding of CRP to antigen-responding T cells has been obtained by studies showing that blastogenesis triggered by antigen (for example, PPD or *Candida*), and the production of release of macrophage migration inhibition factor by human lymphocytes is inhibited by CRP (*9*). Our experiments give some insight into the basis for this selective reactivity. Since CRP reacts with lymphoblasts induced by one but not the other group of stimulating agents, this binding cannot be attributed to changes associated with proliferation per se. Further, although cell injury favors the deposition of CRP (*10*), its binding to dividing as well as to resting cells, and its binding selectively to lymphoblasts induced by one group of agents, indicates that the interaction between CRP and T lymphocytes is not a consequence of cell injury. Since the cell viability was similar in all cultures studied, selective survival of blasts in one set of cultures relative to the others did not seem to influence the results reported. Fi-

Table 1. Percentages of mitogen- and antigen-induced lymphoblasts binding purified human CRP. Washed lymphocytes from the respective cultures were incubated with CRP (50 μg/ml) for 18 hours at 4°C, washed, and reacted with fluoresceinated rabbit antiserum to CRP; the percentages of cells stained ± 1 S.D. are indicated. Viability, as determined by trypan blue dye exclusion, ranged from 76 to 94 percent in all cultures, and no significant differences between the cultures were seen. Cells from four individuals were evaluated for responsiveness to PPD, and from ten individuals for responsiveness to the other agents, each with results similar to those shown in the table; all experiments were repeated at least in triplicate for each lymphocyte donor. The last column shows the ratio of the percentage of blasts stained to the percentage of nonblasts stained.

Mitogen or antigen	Unstimulated cells	Lympho-blasts	Non-lympho-blasts	Ratio of blasts to nonblasts stained
PHA	38.7 (± 2.4)	5.2 (± 1.9)	46.0 (± 7.6)	0.11
Con A	36.3 (± 1.7)	4.1 (± 2.0)	40.7 (± 2.0)	0.10
MLR	36.0 (± 3.0)	22.5 (± 6.9)	41.0 (± 3.6)	0.55
PPD	37.2 (± 4.4)	26.8 (± 2.2)	40.6 (± 2.2)	0.66

nally, because the cells stimulated to divide in vitro by PHA, Con A, antigens, and allogeneic (MLR) cells are known to be predominantly T cells (*11*), selective proliferation of B (bone marrow–derived) cells in response to these mitogens cannot explain the present findings. Instead, our results raise the possibility that CRP reacts with a subpopulation of T cells which responds with proliferation to challenge with soluble or cell surface antigens but not with mitogens. Alternatively, sites capable of binding CRP may become available on the cell surface selectively upon stimulation by antigens as opposed to mitogens.

That the antigen-sensitive T cell may be a distinctive subset has received support from studies showing inactivation of the cells dividing in response to antigen without an effect on the cells capable of responding to PHA, and by separation of cells with these functions by buoyancy gradients (*12*). Thus, although the antigen-sensitive population had been considered by many to be a small proportion of the total pool of T cells (*13*), all of which are potentially responsive to T cell mitogens (*14*), this might not be the case. We do not yet know whether cells responding to blastogenic factors can bind CRP, or whether the lymphoblasts binding CRP are selectively those whose blastogenesis was induced by antigen directly; perhaps recruitment of cells not reactive with CRP explains why only one of four lymphoblasts formed in response to antigenic stimulation was able to bind CRP. A recruitment of cells that do not bind to CRP might also explain the consistently larger proportion of CRP-binding nonlymphoblasts in stimulated compared to unstimulated cultures.

In summary, we report that CRP binds preferentially to lymphoblasts stimulated by antigens as opposed to mitogens. It may thus be possible to use CRP as a marker for antigen- as opposed to mitogen-reactive T cells, or for antigen- as opposed to mitogen-reacted T cells, and to use CRP in the further definition of the surface and functional properties of such cells. While the exact biologic advantage of this interaction is yet to be defined, these experiments support the concept that CRP, by its ability to bind to certain T lymphocytes and influence the functions of T cells (*3, 4, 9*), platelets (*15*), and the C system (*6, 16*), plays an important role in the regulation of a variety of responses critical to inflammation, host defense, and tissue repair.

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Rhizoid Differentiation in Fern Spores: Experimental Manipulation

Abstract. *Germination in spores of the fern* Onoclea sensibilis *is initiated by an asymmetric division that partitions the spore into two cells of unequal size. The unequal daughter cells differentiate immediately into distinct types. When spores are germinated on the surface of solutions of methanol, the initial division is symmetrical, and the daughter cells from this equal division develop into the same type of cell. The differentiation of a rhizoid from the smaller cell in untreated spores is suppressed by methanol treatment.*

Striking examples of cellular differentiation in which two daughter cells—the products of a single mitosis—often diverge immediately and mature into different cell types are known in many plants. Bünning (*1*) reviewed many instances of this phenomenon, including root hair formation and guard cell differentiation in monocotyledons, the development of pollen grains and hyaline cell formation in the leaves of *Sphagnum*, and the germination of fern spores. One important feature shared by all of these systems is that differentiation is initiated with an asymmetric cell division, which partitions the mother cell into two daughters of unequal size. The daughter cells then develop into structurally and functionally different types.

We have discovered a simple technique with which we can almost totally control differentiation in germinating spores of a fern, *Onoclea sensibilis*. The technique is based on controlling whether the first division of the spore is asymmetric or not.

Spores were collected, stored, and sterilized prior to their use (*2*). We sterilized the liquid mediums on which spores were germinated by filtering them through a Millipore filter; aseptic conditions were maintained throughout. We initiated germination by floating spores on the surface of 4 ml of a simple inorganic medium (*2*) contained in culture tubes sealed with screw caps. The spores were exposed to 7000 ± 300 lux white fluorescent light at 27.5° ± 0.5°C. Spores were placed on a microscope slide in a solution of chloral hydrate in acetocarmine stain (*3*), and the details of germination were observed through a microscope. The staining mixture dissolves chloroplasts, which are abundant in spores of *Onoclea*, and stains the nuclei of germinated spores. It is then possible to see the positions of the nuclei and cell walls, which, in untreated spores, are obscured by the chloroplasts.

In ungerminated spores, the nucleus is located in the center. About 16 to 20 hours after germination begins, the nucleus migrates to one end of the spore. Two hours later, mitosis occurs (Fig. 1A), and the spore divides into a large and a small cell (Fig. 1B). The smaller cell narrows and elongates rapidly, differentiating into a rhizoid (Fig. 1, C and D). The rhizoid remains a single, elongated cell and never again divides. The larger cell retains its capacity for division and develops first a filament of two or more cells (the protonema) and later a two-dimensional plate of cells (the prothallus). In the living state, the cells of the protonema contain many chloroplasts whereas the rhi-