Complementation of the plaque-forming cell responses of T-cell-deficient nude mice by a T-cell hybridoma

(cell fusion/mouse T lymphocytes/help T cells)

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ABSTRACT Spleen cells from NFR/N mice primed with carrier rabbit erythrocytes were passed through nylon wool and fused with cells from the AKR T-cell lymphoma BW5147 in order to construct functional T-cell hybridomas. Several hybrids have been obtained. One of the cloned hybrids possesses a marked capacity to functionally complement the T-cell-dependent plaque-forming cell responses of NFR/N nude mice to the trinitrophenyl group. The hybrids carry the Thy 1 marker of the immune parent, express the Lyt 1 surface marker, possess Fe receptors at their surface, and express an Ig heavy-chain variable region determinant at their surfaces as detected by anti-V\textsubscript{H} MOPC 315 antiserum.

The expression of the variable (V)-region gene product of antibody producing B-cells is regulated by other cells of the immune system. Accessory cells such as macrophages and T cells are known to be involved in this regulation. At least two types of T cells are known to be involved; helper T cells exert positive regulation and suppressor T cells exert negative regulation.

We have been attempting to understand how this regulation is mediated at the cellular level. An in vitro cellular complementation system was developed that utilizes the T-cell dependence of hapten-specific plaque-forming cell (PFC) responses of NFR/N mice. This model system involves the regulation of PFC responses to the trinitrophenyl (TNP) group of trinitrophenylated rabbit erythrocytes (TNP-RE). Although T-cell-deficient nude mice cannot respond to T-cell-dependent immunogens, they can be complemented to respond in a way similar to that of normal heterozygous NFR/N +/nu mice by the addition of +/nu T cells (1, 2). This complementing T cell (4) was shown to be in the population of T cells that eluted first from nylon wool columns. We sought to obtain this T-cell subpopulation and other T-cell subpopulations with stable monoclonal phenotypes. Our approach was to attempt to construct functional T-cell hybridomas within the T-cell subpopulations.

In the work reported here we have succeeded in constructing several hybridomas between carrier-primed NFR/N mouse spleen T cells (4) and cells from a mouse T cell lymphoma line. One of these hybridomas, which has been successfully cloned, was observed to have a marked capacity to synergize with NFR/N nude mouse B cells in the generation of a large number of TNP-specific PFC in vitro. The cloned hybrid (BP\textsubscript{1}C\textsubscript{36}) expresses the appropriate T-cell markers consistent with a classification as a T-cell hybridoma.

MATERIALS AND METHODS

Mice. NFR/N nude (nu/nu) mice and NFR/N +/nu mice are obtained on standing order from the Small Animal Section, Division of Research Services, National Institutes of Health.

The NFR/N mouse is an inbred strain of the N:NIH mouse. The inbred NFR/N nude mice are now more than 99.9% homogenous (personal communication, C. Hansen, National Institutes of Health). These mice were derived and are maintained as closed colonies here at the National Institutes of Health as detailed previously (1–6).

Lymphoma Cells. The AKR lymphoma cell line (BW5147 TGr) deficient in hypoxanthine phosphoribosyltransferase (7) was obtained from Nancy Rudde of the Department of Epidemiology, Yale University Medical School, New Haven, CT. A stock of the cells was grown up in Dulbecco’s modified Eagle’s medium containing 20% heat-inactivated fetal bovine serum and preserved in liquid nitrogen. Culturing was seeded and grown from this stock as needed.

Cell Hybridization. Ten million BW5147 cells were mixed with 10⁶ spleen cells obtained from rabbit erythrocyte (RE)-primed NFR/N mice and passed through nylon wool (4). The cell mixture was centrifuged at 1200 x g and the cells were resuspended in 0.2 ml of a 30% solution of M, 1000 polyethylene glycol (Baker) in Dulbecco’s modified Eagle’s medium without serum. Cells were pelleted at 700 x g for 3 min. After the pellet had been in contact with the polyethylene glycol for a total of 5 min, 5 ml of Dulbecco’s modified Eagle’s medium was added without disturbing the pellet. After 1–2 min, the cell pellet was gently resuspended intermittently for 4 min. The cells were centrifuged at 1200 x g for 5 min. The medium was aspirated and replaced with 5 ml of cloning medium (Dulbecco’s modified Eagle’s medium, 10% NCTC-109 (catalog no. 12-123A, M. A. Bioproducts, Walkersville, MD) and 20% fetal bovine serum) without disturbing the pellet. After 7 min, the pellet was gently resuspended and 2.5 ml of the cell suspension was transferred to a tissue culture flask containing 7.5 ml of cloning medium and incubated at 37°C in a tissue culture incubator. The volume of cloning medium was gradually increased 2-fold over a period of 48 hr. At the end of the 48-hr period, the cells were centrifuged at 1200 x g for 10 min and resuspended in HAT cloning medium (cloning medium and 100 μM hypoxanthine, 10 μM aminopterin, and 30 μM thymidine) to a density of 4 x 10⁶ cells per ml, and 0.5 ml was seeded in each well of a 24-well cluster dish (Costar no. 3524). The cultures were incubated at 37°C in an atmosphere of 83% nitrogen/10% CO₂/7% O₂ and 100% relative humidity.

Cloning of Hybrid Cells. Hybrid cells were cloned in soft agar by the following method. A base layer of 0.5% agarose (Sigma no. A6877) in cloning medium was allowed to gel in 60 x 15 mm plastic tissue culture dishes for 10 min at room temperature. The base layer was then overlayed with graded num-

Abbreviations: V, variable region of immunoglobulin; H, heavy chain of immunoglobulin; PFC, plaque-forming cell; TNP, trinitrophenyl; RE, rabbit erythrocyte; SE, sheep erythrocyte; PE, pigeon erythrocyte; Rd, rhodamine; Fl, fluorescein; Mc, monoclonal.
bers of cells from the appropriate clusters from 1.0 to 5.0 x 10^6 cells in 1.0 ml of 0.25% agarose in cloning medium supplemented with 5.5% autologous supernatant fluid from the wells that contained the growing hybrids. The supernatant fluid was made cell free by filtration through a Millipore Millex filter, 0.2-µm pore diameter. After a period of about 11 days incubation, the colonies of hybrid cells were picked with Pasteur pipettes and transferred to 96-well tissue culture cluster dishes (Costar no. 3596) containing 0.2 ml of cloning medium. Those colonies that grew were transferred to 24-well cluster dishes and then grown up in cloning medium in tissue culture flasks.

**Immunogens.** TNP-RE, trinitrophenylated sheep erythrocytes (TNP-SE), and trinitrophenylated pigeon erythrocytes (TNP-PE) were prepared according to the method of Rittenberg and Pratt (8). The optimal number of TNP groups per erythrocyte was empirically determined and was limited by the reaction time and the starting concentration of 2,4,6-trinitrobenzene sulfonic acid in the reaction mixture. Where indicated, mice were primed with carrier by injection of 10^6 RE per mouse intraperitoneally 7 days before use of their spleenocytes.

**PFC Assays.** Estimations of hapten-specific anti-TNP PFC responses of cultures were done in agarose plates as described (2, 3). TNP-SE and TNP-RE prepared by the method of Rittenberg and Pratt (8) were used as target indicator cells. When TNP-RE were used as the target cells, it was helpful to increase the amount of guinea pig complement by 10% and to decrease the number of TNP-RE targets during plating by about 40% relative to standard conditions.

**Karyotype Analysis.** Chromosome counts of the hybrid cells and the fusion partners, BWS147 cells and NFR spleen cells, were performed according to the method outlined in the Gibco chromosome test kit (catalog no. 120-6706).

**Tests for T-Cell Markers by Immunofluorescent Staining.** Direct immunofluorescent staining of the hybrid cells and the fusion partners was performed to test for the T-cell markers Thy 1.2 and Lyt 1. Indirect immunofluorescent staining was used to test for the expression of immunoglobulin heavy chain variable region (VH) determinants by the hybrid cells. Fluorescent cells were scored in the Leitz Ortholux microscope. Twenty million of the appropriate cells were stained with either a directly fluorescein-conjugated rabbit IgG, IgG2a, isotype, or a monoclonal anti-Thy 1.2 (McFl-anti-Thy 1.2), rat IgG2a, isotype (Becton-Dickinson FACS Systems, Monoclonal Antibody Center, Sunnyvale, CA). Purified rat IgG2a and purified rat IgG rich in IgG2a, used as controls were gifts from Jerry McGehee (Department of Microbiology, University of Alabama-Birmingham). These purified non specific immunoglobulins were used as controls for non specific IgG binding by the hybrid T cells. In order to test hybrid cells for VH determinants, 2.0 x 10^6 cells were incubated with a rabbit IgG anti-MOPC 315 VH antibody obtained from David Givol (Weizmann Institute of Science, Rehovot, Israel). Those cells binding the affinity-purified rabbit IgG anti-VH were detected secondarily with a rhodamine-labeled IgG F(ab')2 goat anti-rabbit IgG (Cappel Laboratories, Cochranville, PA).

Other antisera used in control experiments were as follows. Rabbit IgG anti-aaoaspanitale was raised by hyperimmunization of rabbits with p-arsanilic acid conjugated to bovine serum albumin. Goat IgG anti-chicken egg albumin was purchased from Cappel Laboratories. Sheep anti-rabbit IgG was raised in unrelated experiments by hyperimmunization of a sheep with purified rabbit IgG, donated by Rose Mage (National Institutes of Health). This antisera was employed to specifically block rabbit IgG determinants that would be available at the surface of cells after saturation of their Fe rabbit IgG receptors with rabbit IgG. Rhodamine-conjugated rabbit IgG F(ab')2 anti-rat IgG [RD-F(ab')2] rabbit anti-rat IgG, Cappel Laboratories, was used in control experiments to ascertain that rat IgG of the subclasses similar to that of fluoresceinated monoclonal anti-Thy 1.2 and anti-Lyt 1 was not nonspecifically bound by the appropriate cells being tested for Thy 1.2 and Lyt 1 markers. Fluorescein-conjugated rabbit IgG F(ab')2 anti-goat IgG (FL-F(ab')2 rabbit anti-goat IgG), Cappel Laboratories, was used similarly as above to ascertain that goat IgG was not nonspecifically bound to appropriate cells being tested with Rd-F(ab')2 goat anti-rabbit IgG.

**Test for Fc Receptor.** SE obtained from the Division of Research Resources, National Institutes of Health, were washed in Hanks' balanced salt solution. The washed SE were incubated with a 1:2500 dilution of rabbit anti-sheep hemolysin (BBL, Cockeysville, MD) at 37°C for 30 min. Pool I cells, hybrid cells, or fusion partner cells were tested for their capacity to form rosettes with the rabbit IgG-sensitized SE. Rosettes were scored when five or more sheep cells were present around a central lymphocyte.

**Spleen Cell Cultures.** Spleen cells were cultured as detailed before in large Marbrook chambers (1–5) in modified Eagle's medium (α medium) (9) containing glutamine and supplemented with 15% fetal bovine serum. Gentamicin at a concentration of 150 µg/ml was added as antibiotic. Cultures were immunized with 10^6 TNP-RE, TNP-SE, or TNP-PE per culture.

**Complementation Procedure.** The procedure has been detailed (1–4). Briefly, 2.5 x 10^6 ml/ml nu/nu and 1.5 x 10^6 fractionated +/nu spleen cells or 1.5 x 10^6 cultured hybrid cells were mixed and seeded into Marbrook cultures.

**RESULTS**

Fifteen to 20 days after the fusion of nylon wool-passed spleen cells from RE-primed NFR/N mice with BWS147 cells, several wells showing growth were observed. Cells from these wells were isolated, grown, tested for their capacity to complement nude PFC responses, and stored in liquid nitrogen. Those cells that showed the capacity to complement nude PFC responses were cloned in soft agar. In this paper we report results obtained by using three selected cloned hybrids; BP1C36, BP1C37, and BP1C42.

**In Vitro Complementation of the Anti-TNP PFC Responses of Nude Mouse Splenocytes by BP1C42.** The three clones BP1C36, BP1C37, and BP1C42 were tested for their ability to complement nude PFC responses to TNP-RE in the in vitro complementation system. As shown in Fig. 1, BP1C36 was able to complement nude PFC responses to TNP-RE greater than 7-fold in comparison with nude splenocyte cultures alone. BP1C37 could only minimally complement the PFC response of nude splenocyte cultures to TNP-RE; the PFC responses of BP1C37-complemented cultures were only 2-fold the response of nude splenocyte cultures alone. BP1C42 was unable to complement nude splenocyte PFC responses to TNP-RE. BP1C42 was shown to be specific for carrier (RE), because it was able to complement nude splenocyte PFC responses to RE but was unable to complement nude splenocyte PFC responses to the noncrossreacting SE (Fig. 2). The anti-SE PFC responses of cultures complemented with BP1C42 cells were not appreciably greater than the anti-SE PFC responses of nude splenocyte cultures alone, consistent with the specific carrier priming effect. Moreover, carrier specificity was further ascertained by the observation that BP1C36-complemented cultures failed to
be immunized to TNP when TNP was borne on PE or SE carriers (Fig. 3).

Karyotype of Hybrids. Next, it was important to perform karyotype analysis on the clones to ascertain that they were hybrids. Table 1 shows the chromosome number of the hybrids and the fusion partners. BP₁C₃₆, BP₁C₃₇, and BP₁C₄₂ all have more chromosomes than does either parent, but the number of chromosomes is less than the sum of the two parents, indicating that they are hybrids and not heterokaryons or diploids. Being aware of the general problem of karyotype instability in hybrid cells, we have taken the precaution of using only freshly seeded hybrids from liquid nitrogen storage and limiting the number of subcultures.

T-Cell Markers of Hybrids. The next question asked was whether these hybrid cells capable of complementing the anti-TNP PFC responses of NFR/N nude mice bore cell surface markers characteristic of T cells. Table 2 shows the results of experiments performed to determine the presence or absence of selected T-cell markers on the hybrid cells. BP₁C₃₆ cells were observed to express the Thy 1.2 marker and the Lyt 1 marker on their surfaces by immunofluorescence using monoclonal anti-Thy 1.2 and anti-Lyt 1 antisera, respectively. BP₁C₃₆ cells were observed to bind with the affinity-purified rabbit IgG antiserum, obtained from David Givol, which recognizes a V₃₄ fragment. This V region moiety has been proposed as a T-cell receptor (10). We also observe that BP₁C₃₆ cells form rosettes when tested in an EA rosette test using hyperimmune rabbit IgG-sensitized erythrocytes (EA). This observation suggests that BP₁C₃₆ cells possess an Fc receptor (11). BP₁C₄₂ cells have the same surface markers as do BP₁C₃₆ cells, but cells of BP₁C₄₂ were unable to complement nude splenocyte PFC responses. It was consistent that the T lymphoma fusion partner BW5147 cells did not express Thy 1.2, because they were derived from Thy 1.1⁺ AKR mice.

**DISCUSSION**

This paper reports the isolation of a cloned hybridoma with a helper T-cell phenotype and appropriate T-cell markers. This hybridoma line was constructed by the fusion of carrier-primed nylon wool non adherent splenocytes with T-cell lymphoma cells from the BW5147 cell line. This construction was facilitated by our prior establishment of a highly characterized immunocyte complementation system in vitro (1-4). Important to this system is the use of splenocytes from highly inbred NFR/N nude mice as the source of potential antibody-secreting B lymphocytes and of accessory macrophages and the use of splenocytes from NFR/N +/nu mice as a source of regulatory T lymphocytes (1-5). This system is highly reliable for the assessment of the function and phenotype of either T-cell subpopulation as well as the function of other cell types that may augment or suppress the magnitude of in vitro or in vivo PFC responses (1-5).

The hybridoma line BP₁C₃₆ reported in this paper is fully active in complementing the TNP-specific PFC response of the deficiently responding nude mouse B-cell—macrophage population in combination in Marbrook cultures. The TNP-specific
PFC responses of such cultures compared favorably with the responses of cultures that are regularly complemented with freshly fractionated helper T cells (4). The helping activity of BP1C36 exhibited carrier specificity in complementation experiments when BP1C36 was asked to help PFC responses to TNP after culture stimulation with TNP-RE. TNP-specific PFC responses were augmented when they were assayed on TNP-SE targets, indicating TNP specificity. Similar cultures stimulated with RE and assayed on RE target cells for RE-specific PFC showed elevated PFC on RE targets but not on SE targets (Fig. 2). Furthermore, similar cultures stimulated with SE did not show elevated PFC on either target cells (Fig. 2), even though unseparated NFR +/nu spleen cells similar to those from which the pool 1 parent cell of BP1C36 originated were shown to contain native helper T cells for an anti-SE PFC response (1). Moreover, BP1C36 cells were capable of helping an anti-TNP PFC response to TNP-RE immunogen, but BP1C36 cells were incapable of helping an anti-TNP PFC response to TNP-SE or TNP-PE (Fig. 3). Thus carrier specificity for RE was observed in the helping activity of BP1C36.

We observe that BP1C36, as well as the two other hybrids tested to date, BP1C37 and BP1C42, is hyperploid as expected of a hybrid cell (Table 1). They were observed to have high, low, and negative capacities, respectively, to complement—i.e., help nude mouse PFC responses to TNP.

With respect to cell surface markers on the hybrids that are characteristic of helper T cells, we detected the presence of the product of the Thy 1.2 allele. This allele was probably derived from the NFR immune cell parent, because the AKR lymphoma parent is Thy 1.1. We cannot rule out the expression of both alleles on any of our hybrids because we have in our possession only an anti-Thy 1.2 monoclonal antisera. Reports from other laboratories have indicated coexpression of both allelic products (Thy 1.1 and Thy 1.2) on other types of T-cell hybridomas (12–14). The detection of the V\textsubscript{H} determinant (10) on BP1C36 and BP1C42 is interesting in two respects. These hybrids had been grown for several generations outside of animal hosts, therefore the V\textsubscript{H} determinant is likely to be a gene product of the T cell (hybrid) and not a passively conferred B-cell product. It should be pointed out that in our hands the malignant lymphoma parent BW5147 also appeared to express the V\textsubscript{H} gene product at its surface, although there appeared to be less of it present on these cells than was present on the hybrids. Noteworthy is the fact that we also detected an Fc receptor present on hybrids BP1C36 and BP1C42 and on pool 1 cells (Table 2). This observation suggested that our hybrid T cells may express two separate gene products capable of being T-cell receptors. It was important to ascertain that the rabbit IgG anti-mouse V\textsubscript{H} was not passively bound (Fc bound?) by BP1C36 cells. It was also important to ascertain that the monoclonal antisera, anti-Thy 1.2 and anti-Lyt 1, were not passively bound. Our approaches to both of these problems were similar. In the approach to the first problem BP1C36 cells were incubated with an excess of an irrelevant rabbit IgG antisera (anti-arsanilic acid) prior to testing them for their capacity to bind rabbit anti-V\textsubscript{H} antisera (Table 3). Because the bound rabbit anti-V\textsubscript{H} is detected secondarily by a rhodamine-labeled goat IgG F(ab')\textsubscript{2} anti-rabbit antisera, the BP1C36 cells were also preincubated with an excess amount of an irrelevant goat IgG antisera (anti-chicken egg albumin). The results showed that BP1C36 cells bound the rabbit IgG anti-V\textsubscript{H} even after incubation with excess rabbit IgG of irrelevant specificity. Note that IgG determinants present on the irrelevant rabbit IgG were blocked by use of a sheep anti-rabbit IgG antisera prior to incubation of the cells with the rabbit anti-V\textsubscript{H} antisera. This was done to ensure that the final addition of Rd-F(ab')\textsubscript{2} goat anti-rabbit IgG would detect only

<table>
<thead>
<tr>
<th>Cell surface markers</th>
<th>Cell</th>
<th>Thy 1.2</th>
<th>Lyt 1</th>
<th>Fc receptor</th>
<th>V\textsubscript{H} marker</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP1C36</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>BP1C37</td>
<td>+</td>
<td>+</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>BP1C42</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>NFR/N pool 1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>BW5147</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>±</td>
<td></td>
</tr>
</tbody>
</table>

ND, measurement not done.


Table 3. Specificity of the binding of affinity-purified rabbit IgG anti-mouse V\textsubscript{H}, (MOPC-315) to cells of clone BP1C36

<table>
<thead>
<tr>
<th>Preincubation of cells with IgG*</th>
<th>Second incubation of cells with relevant antisera</th>
<th>Final incubation with Rd-Fab\textsubscript{2} goat anti-rabbit IgG</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>None</td>
<td>Amount, μl</td>
</tr>
<tr>
<td>None</td>
<td>None</td>
<td>20</td>
</tr>
<tr>
<td>None</td>
<td>Rabbit anti-mouse V\textsubscript{H}, 3 μg</td>
<td>10</td>
</tr>
<tr>
<td>Rabbit + goat IgG, 1:5 dilution, 50 μl</td>
<td>Same, 6 μg</td>
<td>5</td>
</tr>
<tr>
<td>Same</td>
<td>None</td>
<td>10</td>
</tr>
<tr>
<td>Same</td>
<td>Sheep anti-rabbit IgG, 1:5 dilution, 100 μl</td>
<td>10</td>
</tr>
<tr>
<td>Same</td>
<td>Same + rabbit anti-mouse V\textsubscript{H}, 6 μg</td>
<td>10</td>
</tr>
</tbody>
</table>

*Cells (2 × 10\textsuperscript{6} per 100 μl) were treated with 1% paraformaldehyde in Hanks' balanced salt solution containing 3% heated fetal bovine serum, washed, and maintained in Hanks' solution plus 3% heated fetal bovine serum. All incubations were at room temperature for 30 min followed by washing twice in 100 vol of Hanks' solution plus 3% heated bovine serum.

† –, No visual evidence of cells under incident fluorescence excitation of the same field in which cells were observed by dark-field phase; ×, very weakly fluorescing cells, barely visible; +, definite fluorescence of cells.
the bound rabbit anti-V\textsubscript{H} (Table 3). The fluorescence of the cells in the final step of this procedure corroborates the specific binding of the anti-V\textsubscript{H} antisera by BP\textsubscript{1}C\textsubscript{36} hybrid cells as observed (Table 2).

Relative to the second problem, the monoclonal anti-Thy 1.2 and anti-Lyt 1 are products of mouse–rat hybridomas. Anti-Thy 1 is an IgG\textsubscript{2a} H chain isotype and anti-Thy 1.2 is an IgG\textsubscript{1}a H chain isotype. BP\textsubscript{1}C\textsubscript{36} cells were incubated with an excess of purified rat IgG\textsubscript{1a} and an excess of purified IgG rich in IgG\textsubscript{2a}. The cells were next incubated with the fluorescein-conjugated monoclonal antibodies anti-Thy 1 and anti-Thy 1.2 and tested for fluorescence. The results showed that BP\textsubscript{1}C\textsubscript{36} cells bound the monoclonal fluorescein-conjugated anti-Thy 1 and anti-Thy 1.2 even after preincubation with excess purified rat IgG of the homologous subclasses of irrelevant antibody specificity (Table 4). Moreover, the data suggested that BP\textsubscript{1}C\textsubscript{36} may not express an Fc receptor for rat IgG, because no fluorescence was observed on BP\textsubscript{1}C\textsubscript{36} cells preincubated with excess rat IgG and then with Rd-F(ab\textsuperscript{'})\textsubscript{2} rabbit anti-rat IgG (Table 4). These results corroborate the specific binding of the monoclonal antibodies by BP\textsubscript{1}C\textsubscript{36} cells (Table 2).

Interestingly, we observed hybrids—e.g., BP\textsubscript{1}C\textsubscript{46}—that bore T-cell markers but were apparently devoid of T-cell helper function (Table 2, Fig. 1). This result, along with the results of preliminary tests of several of the other potential T-cell hybrids constructed, suggests that we may achieve isolation or “immortalization” of the major array of T-cell phenotypes. These clones of cells will be useful in ascertaining the multiple roles of T cells and their mechanisms in the regulation of the immune system. This approach may lead to a better understanding of T-cell circuitry (15, 16), because the stabilization of functions by use of monoclonal cell lines would foster the study of the genetic machinery encoding the phenotypes.

A library consisting of an array of immune cells with various phenotypes should provide discrete targets for understanding the roles of various hormonal and environmental influences on the immune system. The roles of the several mitogens of microbial and plant origins are of major interest in this laboratory and were important in the design of this approach.

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