that received treatment as late as 13 days after tumor dissemination survived >173 days. Unlabeled B4 was minimally active in mice (n = 5 per group) with median survival of 44 or 40 days for mice treated with 0.002 or 0.20 mg, respectively. Untreated controls (n = 15) had a median survival time of 28 days. Therefore, in this lymphoma model, although specificity and dose level were important factors in efficacy, the delay between tumor dissemination and initiation of treatment was less relevant up to a certain time point, at which it was then inversely related to activity. This observation may be related to the geometry of the emitted radiation of the alpha particle, which may kill a cluster of cells more easily than a single cell.

Previous workers have concluded that therapy with ^{225}Ac-constructs might not be feasible because the constructs are unstable and because the radionuclidic daughters present an untenable pharmacological problem (*21–24*). However, our findings indicate that ^{225}Ac can be used as a safe and potent tumor-selective molecular-sized generator in both established solid carcinomas or disseminated cancers. In part, the enhanced potency of these constructs as compared to the ^{213}Bi analogs can be attributed to the longer half-life (313-fold greater ^{225}Ac half-life) and the four net alpha particles emitted by the ^{225}Ac, but other mechanisms must also be involved, such as more efficient cytotoxicity following intracellular delivery of the generator. Once inside the cell, the geometry of the decay trajectory of the alpha particle favors highly efficient cell killing: each decay must pass through the cell, whereas statistically only 30% of the alpha decays will pass through the cell if the generator is surface bound (*2*). Selection of tumor antigen systems that internalize the ^{225}Ac generator construct help to retain the daughters and therefore lead to enhanced potency; however, internalization is not required for activity.

The development of synthetic methods to yield stable nanogenerator constructs of [^{225}Ac]IgG in useful quantities, and the demonstration of safe, efficacious deployment against murine models of both solid carcinomas and disseminated cancer, using very small doses of isotope, suggest a pathway to widespread clinical use of such targeted drugs. The 10-day half-life of the ^{225}Ac generator constructs would allow the drugs to be manufactured at a central radiopharmacy and shipped throughout the world. Because of the extraordinary potency of ^{225}Ac generators, little radioactivity [possibly sub-MBq (mCi)] would be required for therapeutic human use, allowing for economical outpatient use and safety. In addition, the longer half-life of ^{225}Ac may allow better penetration of larger tumors.

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25. Supported by NIH grants RO1 CA55349 and PO1 33049, the CaPCure Foundation, the Lymphoma Foundation, the Cure For Lymphoma Foundation, the Gabrielle Rich Foundation, the Doris Duke Charitable Foundation, and the Pepsico Foundation. We thank M. Geerlings Sr. for helpful discussions.

5 July 2001; accepted 27 September 2001

Induction of Dendritic Cell Differentiation by IFN-α in Systemic Lupus Erythematosus

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Dendritic cells (DCs) are important in regulating both immunity and tolerance. Hence, we hypothesized that systemic lupus erythematosus (SLE), an autoimmune disease characterized by autoreactive B and T cells, may be caused by alterations in the functions of DCs. Consistent with this, monocytes from SLE patients' blood were found to function as antigen-presenting cells, in vitro. Furthermore, serum from SLE patients induced normal monocytes to differentiate into DCs. These DCs could capture antigens from dying cells and present them to CD4-positive T cells. The capacity of SLE patients' serum to induce DC differentiation correlated with disease activity and depended on the actions of interferon-α (IFN-α). Thus, unabated induction of DCs by IFN-α may drive the autoimmune response in SLE.

Lupus (SLE) is a systemic autoimmune disease characterized by a waxing and waning course and the involvement of multiple organs, including skin, kidneys, and central nervous system (*1*). Although SLE etiopathology is poorly understood, there is likely a role for environmental triggers, for instance viruses, acting in the context of susceptibility genes (*2*, *3*). Now, SLE therapy is based on nonspecific immunosuppression and symptom control; however, no cure for SLE has been found, and that is particularly needed for children, whose disease often progresses to chronic renal failure and/or death (*1*). Among the immunological features of SLE are high titers of autoantibodies predominantly specific for DNA and nucleosomes (*4*, *5*). Paradoxically, polyclonal hypergammaglobulinemia, high plasma cell numbers, and an increased frequency of pre–germinal center B cells (*6*, *7*) coincide with considerable B lymphopenia. Likewise, autoreactive T cells are found in the blood despite T lymphopenia (*8*, *9*). The systemic autoimmune response that characterizes SLE might be explained by alter-

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ations in the function of dendritic cells (DCs), because these cells are key regulators of the immune system. DCs are professional antigen-presenting cells (APCs) capable of inducing activation of naïve T cells (*10*, *11*) and of stimulating B cell growth and differentiation (*12*, *13*). We therefore investigated whether blood from pediatric SLE patients displayed any abnormalities in DC function.

We found that $CD11c^+$ myeloid DCs (mDCs) were markedly decreased in SLE blood [~44% decrease (*14*)] but not in a control group of patients with juvenile dermatomyositis (JDM) ($P = 0.1$) who undergo a similar regimen of steroid therapy (*14*). Monocytes, which are the precursors of mDCs, were only 20% decreased (*14*, *15*). Although normal monocytes are not efficient stimulators of naïve allogeneic $CD4^+$ T cell proliferation [mixed lymphocyte reaction (MLR)], those derived from a proportion of SLE patients were able to induce strong MLR (Fig. 1A), a property that can normally be used to define DCs (*16*). This led us to the hypothesis that SLE patients' serum might favor differentiation of monocytes into DCs. Consistent with this, exposure of normal monocytes to such serum resulted, within 24 to 72 hours, in the clustering and formation of cells with morphology typical of DCs (Fig. 1B). These cells displayed a pattern of surface markers consistent with mature DC differentiation, including a down-regulation of CD14, along with an increased expression of major histocompatibility complex (MHC) class II, costimulatory molecules CD80 and CD86, and CD83 (Fig. 1C). Furthermore, normal monocytes cultured with SLE serum induced a strong MLR, whereas those cultured with autologous serum did not (Fig. 1D) (*16*). When serum samples from 20 different patients were tested for DC-inducing activity (*16*), 12 of them generated DCs able to induce proliferation of allogeneic T cells (*17*). Meanwhile, other samples of allogeneic serum from eight healthy controls and from patients with other systemic autoimmune diseases (i.e., JDM and juvenile arthritis, $n = 5$) were unable to do so (*17*). This capacity to induce DC differentiation correlated with disease activity according to SLEDAI (SLE disease activity index; $P = 0.03$; Fig. 1E) (*16*). Finally, normal monocytes cultured with SLE patients' serum, but not those cultured with autologous serum, were able to capture dying allogeneic cells (Fig. 2, A and B) and to present their antigens to autologous normal $CD4^+$ T cells leading to their proliferation (Fig. 2C) (*15*). Thus, morphology, phenotype, and antigen-presenting capacity indicate that serum from SLE patients induces monocytes to differentiate into DCs. It is noteworthy that, in the context of SLE, these DCs present antigens from captured apoptotic cells (*18*).

We next investigated the nature of the SLE serum factor(s) that might cause monocyte differentiation into DCs. Several cytokines can induce the differentiation of monocytes into DCs including granulocyte/macrophage colony-stimulating factor (GM-CSF), interleukin 4 (IL-4), IFN-α, and CD40-L (*11*). Among those, relatively large amounts of IFN-α and CD40-L have been found in the serum of many SLE patients, and their titers correlate with the titers of anti-DNA antibodies and disease activity (*19*–*22*). As shown in Fig. 3A, DC induction, determined by the capacity of normal monocytes cultured with SLE patients' serum to stimulate MLR, was inhibited by neutralizing antibodies against IFN-α (*23*). In contrast, blocking CD40-L, IL-4, or GM-CSF with relevant antibodies did not alter the DC-inducing capacity of serum from SLE patients (*14*). These results point to IFN-α as the major factor in SLE serum that mediates monocyte differentiation to DCs. Accordingly, the addition of IFN-α to normal serum induced monocytes to become potent stimulators of MLR (Fig. 3B) (*23*). Finally, DC induction correlated with increased IFN-α in serum ($P < 0.001$) (Fig. 3C) (*23*). Consistent with this, the ability of monocytes derived from SLE patients to stimulate MLR correlated with IFN-α in serum as well (*17*).

Finally, we investigated the cellular source of IFN-α in SLE patients' blood. Potentially, IFN-α could originate from $CD11c^-$ $CD123^+$ plasmacytoid DCs (pDCs), a unique subset of DCs, which are a major source of IFN-α in the blood (*24*). Unexpectedly, pDC numbers were markedly reduced in the blood of SLE patients (Fig. 4A). This may have reflected an accelerated migration of pDC

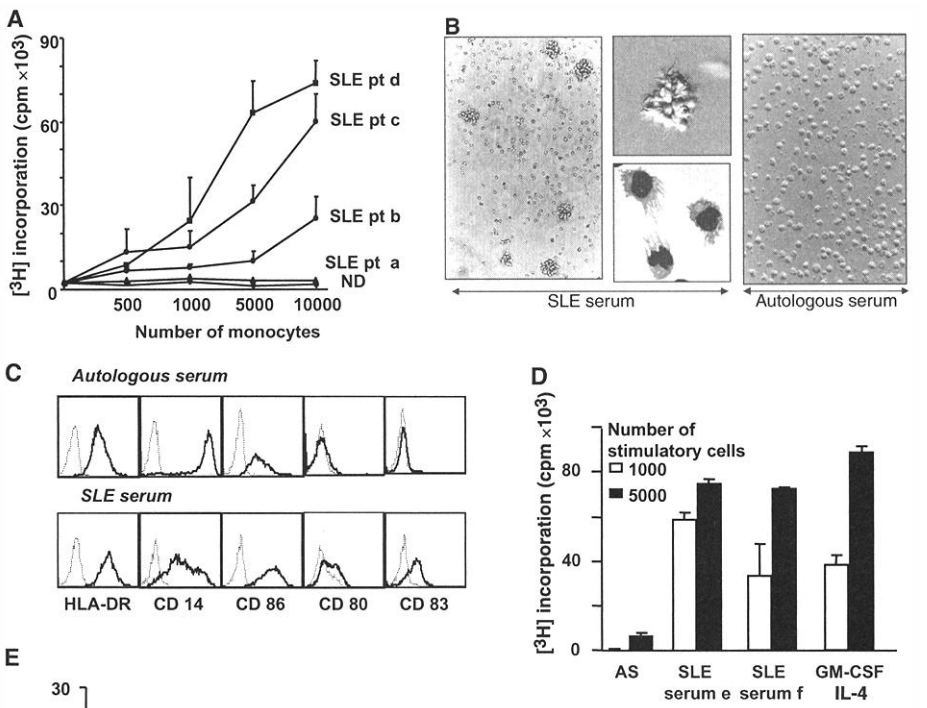

Fig. 1. (**A**) Monocytes from a proportion of SLE patients induce proliferation of allogeneic $CD4^+$ T cells. Lowercase letters refer to patients. (**B**) SLE patients' serum induces monocytes to differentiate into cells with DC properties. Monocytes cultured with SLE patients' serum (left), but not with autologous serum (right), cluster within 12 to 24 hours, and, within 24 to 72 hours, clustered cells display fine cytoplasmic projections (Giemsa staining, middle). (**C**) Flow cytometry analysis of surface markers. (**D**) Monocytes cultured with serum from SLE patients induce proliferation of naïve $CD4^+CD45RA^+$ T cells. Representative of three experiments. (**E**) Induction of DC differentiation is related to disease activity. Monocytes cultured with 20 different samples of serum from SLE patients were tested for induction of T cell proliferation. GM-CSF and IL-4 cultured monocytes were used as a 100% "gold standard." The allostimulatory activity of monocytes cultured with SLE patients' serum is expressed as a percentage of T cell proliferation induced by GM-CSF/IL-4 monocytes (horizontal axis) and related to disease activity (SLEDAI, vertical axis). SLEDAI consists of nine groups of clinical and laboratory criteria that include assessment of organ systems: CNS, vascular, renal, musculoskeletal, serosal, dermal, immunologic, and hematologic. Simple regression analysis ($r^2 = 0.23$).

from the blood into tissues (*25*). Although low in numbers, the CD123$^+$ cells isolated from SLE patients' blood produced normal levels of IFN-α in response to viral triggering (Fig. 4B). We also examined whether pDCs were the only source of IFN-α in SLE patients. Depletion of CD123$^+$ cells from normal peripheral blood mononuclear cells (PBMCs) resulted in a 95% reduction of IFN-α release upon viral triggering (Fig. 4C). In contrast, depletion of CD123$^+$ cells from PBMCs from SLE patients resulted in an average 57% reduction of IFN-α release upon viral triggering (Fig. 4C). Thus, other cells within PBMCs are capable of IFN-α production in SLE.

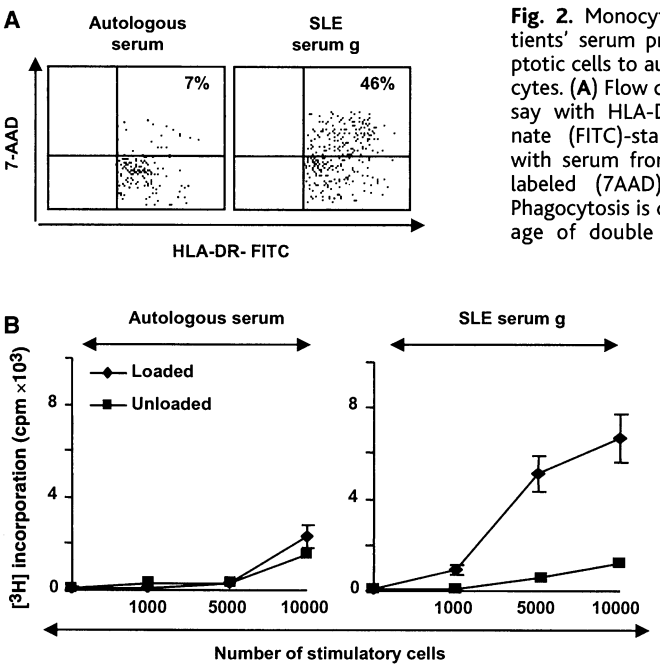

Fig. 2. Monocytes cultured with SLE patients' serum present antigens from apoptotic cells to autologous CD4$^+$ T lymphocytes. (**A**) Flow cytometry phagocytosis assay with HLA-DR fluorescein isothiocyanate (FITC)-stained monocytes cultured with serum from SLE patients and DNA-labeled (7AAD), killed allogeneic cells. Phagocytosis is determined as the percentage of double fluorescence. (**B**) Monocytes cultured with SLE patients' serum present antigens from apoptotic cells to autologous CD4$^+$ T cells as indicated by cell proliferation (means ± SD from triplicates). Representative of three experiments.

In conclusion, patients with SLE display major alterations in DC homeostasis. Whereas one DC subset, i.e., pDCs, is dramatically reduced in the blood, normally quiescent monocytes act as DCs in these patients. This is caused by circulating IFN-α that induces monocytes to differentiate into DCs. Such DCs might very efficiently capture apoptotic cells and nucleosomes, present in SLE patients' blood (*26*, *27*). Subsequent presentation of autoantigens to CD4$^+$ T cells could thus initiate the expansion of autoreactive T cells, followed by differentiation of autoantibody-producing B cells. Autoantibodies could form immune complexes with circulating nucleosomes, which could potentially sustain IFN-α production (*28*). High circulating IFN-α could also explain the puzzling T and B lymphopenia found in SLE (*29*) and the presence of CD38$^+$ pre–germinal center B cells in SLE patients' blood (*7*, *30*). Taken

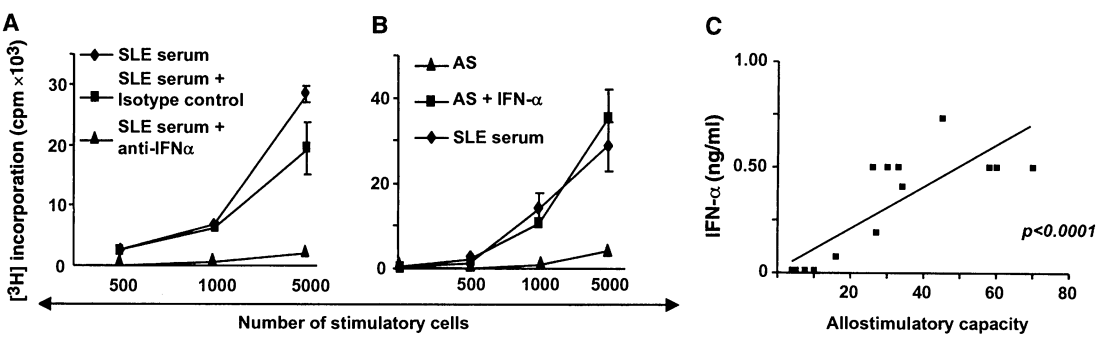

Fig. 3. Induction of DC differentiation is IFN-α dependent. (**A**) IFN-α neutralizing antibody (10 μg/ml) blocks the induction of DCs by SLE patients' serum. Representative of three experiments. (**B**) Spiking autologous serum with 100 UI/ml IFN-α results in the induction of monocyte differentiation to DCs as determined by MLR capacity. (**C**) The capacity of monocytes cultured with serum from SLE patients to stimulate MLR is expressed as in Fig. 1E and related, in simple regression (r^2 = 0.70), to the serum levels of IFN-α (range of ELISA: 12.5 to 500 pg/ml).

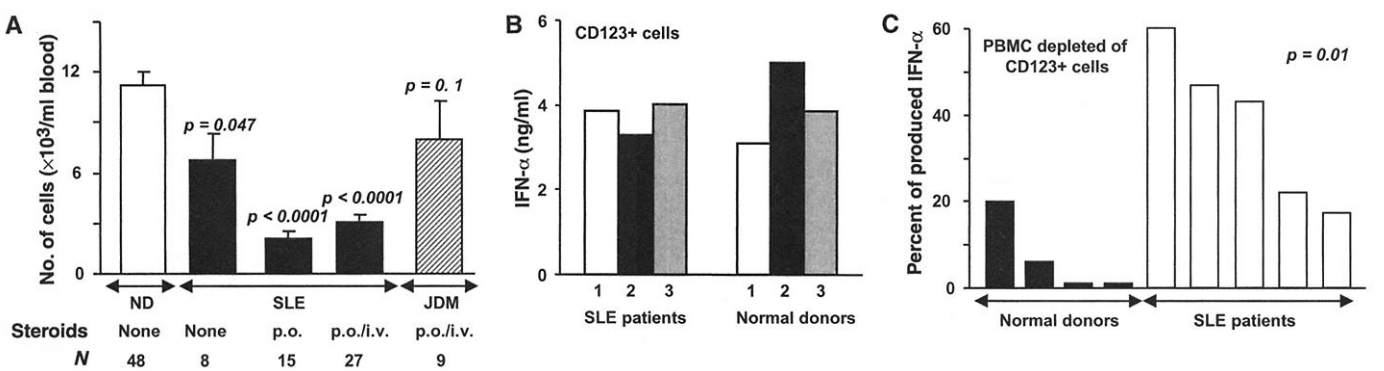

Fig. 4. (**A**) Pediatric SLE patients have lower numbers of HLA-DR$^+$CD11c$^-$ CD123$^+$ pDCs. Numbers of pDCs × 10^3/ml blood (means ± SEM; *t* test). (**B**) CD123$^+$ cells from either normal blood or SLE blood release similar amounts of IFN-α after stimulation with influenza virus [multiplicity of infection (MOI) of 2]. (**C**) PBMCs from SLE patients retain the capacity to produce IFN-α after depletion of CD123$^+$ cells. PBMCs from four normal donors and from five SLE patients, either total or depleted of CD123$^+$ cells (by flow sorting), were cultured overnight with influenza virus (MOI 2). Percentage of IFN-α production reflects the IFN-α produced by PBMCs depleted of CD123$^+$ cells as compared with the amounts from total PBMCs (unpaired *t* test on log-transformed data and nonparametric Mann-Whitney test).

together, our results suggest that IFN-α might represent a potential target for therapeutic intervention in SLE.

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15. After informed consent (IRB no. 0199017), blood was obtained from pediatric patients who satisfied diagnostic criteria of the American College of Rheumatology (ACR) for SLE (median age = 15; range: 7 to 18) and controls (children visiting the clinic for reasons other than autoimmunity or infectious disease, median age = 12; range: <1 to 17), and from age-matched patients with dermatomyositis or juvenile arthritis. Subsets of DCs and monocytes were determined using DC kit and/or CD14 monoclonal antibody (mAb) (Becton Dickinson). Disease activity was assessed by the SLEDAI determined the day of blood draw. SLE patients were evaluated in three groups on the basis of the steroid regimen: (i) either newly diagnosed or not on steroid regimen [neither intravenous (i.v.) pulse nor oral (per os p.o.)]; (ii) not on steroid pulse (but on p.o. dose); or (iii) the blood sample was taken a minimum of 2 weeks after the last steroid pulse (>2 weeks post), and the median time post-pulse was 4 weeks (range 2 to 52 weeks). The remaining treatment included Cytoxan and/or Plaquenil. Patients with JDM were on a steroid regimen (both pulse and p.o. doses), and the blood was evaluated at a median of 6 weeks post pulse (range 2 to 52 weeks). The remaining treatments included methotrexate and/or Plaquenil.
16. $CD14^+$ monocytes were purified from blood by depletion using monoclonal antibodies and Dynabeads and were cultured in six-well plates (1×10^6/well) for 3 days with GM-CSF (100 ng/ml) + IL-4 (20 ng/ml) or lupus serum (25%) or autologous serum (25%). On day 3, cells were stained for flow cytometry. For proliferation assays, DCs were cultured at graded doses with 1×10^5 total $CD4^+$ or naïve $CD4^+CD45RA^+$ allogeneic T cells for 5 days. Cells were pulsed with 0.5 mCi [^{3}H]thymidine per well (NEN). The antigen capture activity was determined by incubating the human lymphocyte antigen HLA-DR–labeled DCs with COLO829 melanoma cell line killed by γ-irradiation (150 Gy) labeled with DNA dye 7-AAD (SIGMA) for 1 hour at 37°C (or on ice as a control). The capture was analyzed by flow cytometry. To assay autologous T cell proliferation, DCs were loaded with killed cells for 4 hours and cultured at graded doses with 1×10^5 $CD4^+$ T cells for 5 days.
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31. Supported by grants from the Baylor Health Care System Foundation; ARC, "Centre Hospitalo-Universitaire de Bordeaux" to P.B., and NIH grant R01 AR46589 to V.P. We would like to thank E. Arce, N. Taquet, E. T. Kraus, S. Coquery, and K. Brooks for help, and S. Koutouzov for discussion.

31 July 2001; accepted 11 October 2001

Choice Behavior of *Drosophila* Facing Contradictory Visual Cues

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We studied the underlying neural mechanism of a simple choice behavior between competing alternatives in *Drosophila*. In a flight simulator, individual flies were conditioned to choose one of two flight paths in response to color and shape cues; after the training, they were tested with contradictory cues. Wild-type flies made a discrete choice that switched from one alternative to the other as the relative salience of color and shape cues gradually changed, but this ability was greatly diminished in mutant (*mbm[1]*) flies with miniature mushroom bodies or with hydroxyurea ablation of mushroom bodies. Thus, *Drosophila* genetics may be useful for elucidating the neural basis of choice behavior.

Most animals can make a rapid and rational choice among alternative behaviors by assessing the advantages and disadvantages on the basis of previous experience, but the underlying neural mechanism is largely unknown (*1*). Studies of simple organisms have made important contributions to our understanding of the cellular and molecular basis of learning and memory, as well as other cognitive functions (*2–5*). Here, we used the visual learning paradigm developed by Wolf and Heisenberg (*6*) to examine choice behavior in *Drosophila*. Individual flies were presented with two conflicting cues, which they had been previously conditioned to follow, and their choice behavior was examined.

An individual fly in a flight simulator was trained to associate a particular visual pattern with a punishment (heat). In a typical protocol, the fly was first examined during a test period (three 2-min blocks) for its directional preference for various patterns in the flight arena. This was followed by two training sessions (two 2-min blocks each, spaced by one 2-min test block) during which the heat was switched on whenever a particular pattern entered the frontal 90° sector of the fly's visual field. The posttraining test sessions consisted of four 2-min blocks without heat application. In experiments in which wild-type Berlin (WTB) flies (*5*) were trained to follow a color cue (*7*), the flies showed no color preference between a green T and a blue T on a dark background during the pretraining test (preference index $PI_{1-3} \sim 0$) (*6*) (Fig. 1A). After the training to associate the heat punishment with the blue T, they exhibited persistent preference for the green T (positive PI_{9-12}). The flight path angular histograms in Fig. 1B (average of 26 flies) depict, at 0.5-min intervals, the relative amount of time spent by the flies in different directions between –90° and +90° relative to the location of the green T. However, when the same experiment was carried out with colored Ts on a white background, the flies failed to learn a preferred color T (Fig. 1C). White background illumination may result in a loss of color sensitivity of fly photoreceptors (*8*); thus, a dark background was used in all experiments. In the absence of color cues, the WTB flies could also learn to choose correctly between a white upright T and an inverted T (Fig. 1D). Thus, WTB flies can use either color or shape cue alone in visual learning.

The *Drosophila* eye is differentially sensitive to green and blue light (*9*). We made

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