We do not know whether nonresponsiveness to the noninherited maternal class II antigens also exists. We found no evidence that a DR incompatibility on the not inherited maternal haplotype increases the chance to develop nonresponsiveness toward the maternal class I antigens, as is suggested in neonatal tolerance in the mouse (*12*).

Our findings raise a number of questions both of a fundamental and a practical character. For instance, we do not know whether what holds true for antibody formation also holds true for T cell activation. It is also unknown whether patients who are not highly sensitized can be nonresponders against NIMA; that is, whether sensitization plays a role in activating a (latent) nonresponder status.

The guidelines for selecting organ or bone marrow donors should perhaps be reconsidered. Fifteen of the 26 patients studied showed B cell unresponsiveness against the NIMAs; therefore, about half of the patients mismatched for one (or sometimes two) HLA class I (and maybe also class II antigens), if these are identical to the NIMA, may have graft survival similar to that of HLA identical grafts. Thus a larger percentage of patients could be provided with an organ or a bone marrow transplant with a better than average prognosis.

It may now be insufficient to ask whether a disease, X, is significantly associated with a certain HLA antigen, Z, in the patient. Patients suffering from disease X, and who are HLA-Z negative, should be checked for tolerance to a NIMA. It then still remains to be established whether Z is necessarily the tolerating antigen. Such a hypothesis might be supported by observations that show a significantly decreased frequency of HLA-B5 in the mothers of patients suffering from rheumatic fever (*13*). Also, experiments in the mouse indicate that neonatal tolerance to MHC antigens alters the Ir gene control of the cytotoxic T cell response to vaccinia virus (*14*).

In conclusion, the high frequency of NIMAs among the permissible mismatches of highly sensitized patients suggests that the fetus cannot only immunize its mother (*15, 16*), but that the mother can also induce partial, but lifelong, tolerance in some of her children. Further studies should reveal whether the nonresponsiveness is due to clonal deletion, suppressor cells, or other mechanisms.

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Hyperthermia Protects Against Light Damage in the Rat Retina

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An increase in the synthesis of heat shock proteins that is induced in cells in vitro by hyperthermia or other types of metabolic stress correlates with enhanced cell survival upon further stress. To determine if a similar increase in stress tolerance could be elicited in vivo, rats were made hyperthermic, and then their retinas were tested for sensitivity to light damage. This treatment resulted in a marked decrease in photoreceptor degeneration after exposure to bright light as compared to normothermic animals. Concomitant with such protection was an increase in retinal synthesis of three heat shock proteins. Thus, a physiological rise in body temperature enhances the stress tolerance of nerve tissue, perhaps by increasing heat shock protein production.

MANY ORGANISMS PRODUCE A family of "heat shock" or "stress" proteins (HSPs) after hyperthermia (*1–3*). The production of HSPs correlates with the acquisition of tolerance to a wide variety of stressors in cultured cells and tissues (*2, 4–7*). For example, exposure of cells to a mild heat challenge greatly enhances cell survival after a subsequent, and what would otherwise be a lethal, elevation in temperature (*4–7*). This protective effect, referred to as acquired thermotolerance, is correlated with the expression of the HSPs. In the central nervous system (CNS), the stress response may be of crucial importance to the outcome of injury if it is involved in cell survival. This response is a general feature of CNS reaction to injury, since HSP production is elevated by D-lysergic acid diethylamide (LSD) (*8*), hyperthermia (*9, 10*), ischemia (*11*), sodium arsenite (*12*), surgical cutting (*13*), and concussive injury (*14*). We have begun to explore this response by using the retina as a model for the CNS. Prolonged or excessive exposure of the retina to light results in irreversible damage to the photoreceptor cells (*15–18*). If a mild heat stress in vivo also leads to enhanced stress tolerance, then perhaps prior hyperthermia in the rat could reduce death of photoreceptor cells caused by subsequent exposure to bright light, and thus reduce damage to the retina. We report here that a brief period of hyperthermia before light exposure significantly reduced photoreceptor degeneration while it stimulated HSP production.

We and another group have shown that production of one or more HSPs can be stimulated in the retina after a brief period of heat stress and that the pattern of these newly synthesized retinal HSPs as revealed by two-dimensional gel electrophoresis was very similar to that reported in other systems (*9, 10*). This procedure, combined with a well-established protocol for quantitation of light damage in the rat retina (*15–17, 19*),

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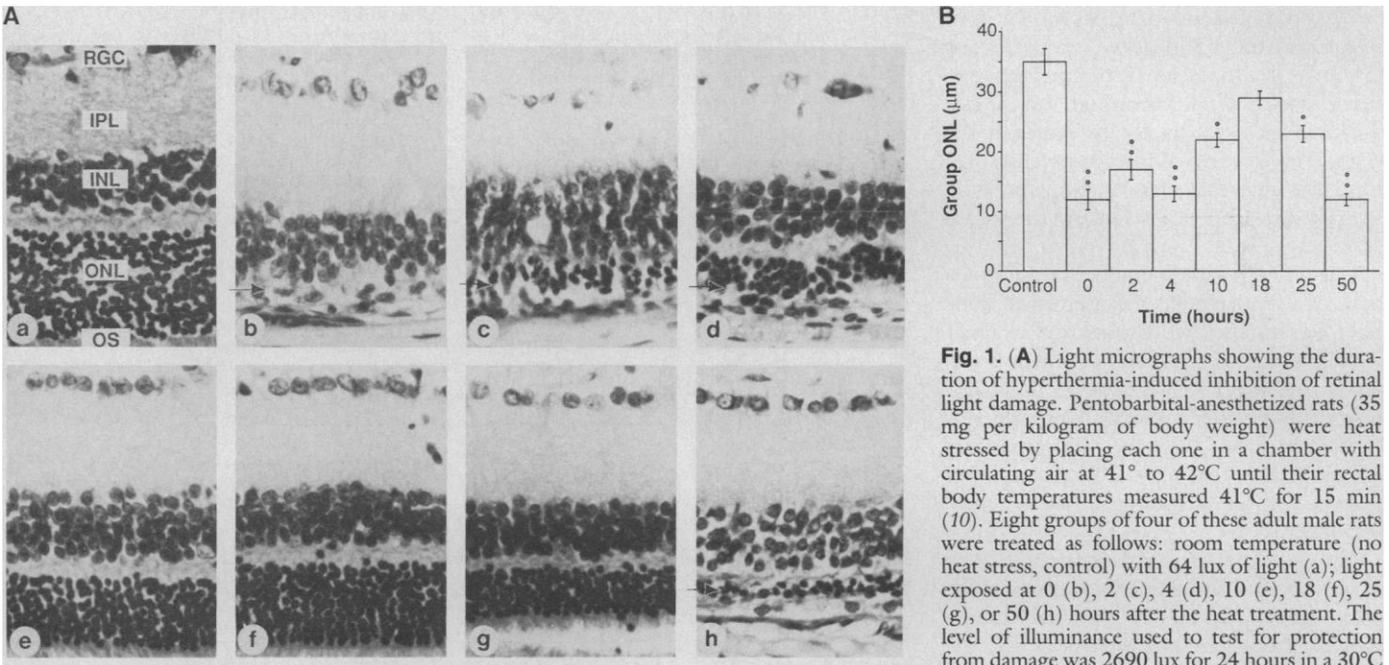

Fig. 1. (**A**) Light micrographs showing the duration of hyperthermia-induced inhibition of retinal light damage. Pentobarbital-anesthetized rats (35 mg per kilogram of body weight) were heat stressed by placing each one in a chamber with circulating air at 41° to 42°C until their rectal body temperatures measured 41°C for 15 min (*10*). Eight groups of four of these adult male rats were treated as follows: room temperature (no heat stress, control) with 64 lux of light (a); light exposed at 0 (b), 2 (c), 4 (d), 10 (e), 18 (f), 25 (g), or 50 (h) hours after the heat treatment. The level of illuminance used to test for protection from damage was 2690 lux for 24 hours in a 30°C chamber (*17*). All rats were killed after 2 weeks of being kept under control conditions. The eyes were removed, placed in Bouin's fixative for 5 hours, and routinely processed for paraffin embedding and light microscopy. Retinas were sectioned on the anterior-posterior axis. The retinas were stained with hematoxylin and eosin, and all micrographs were made at a magnification (×400) of retinal area 4, which is 2 mm from the peripheral edge of the superior temporal retina, as defined in (*19*). The retinal layers were labeled as follows: RGC, retinal ganglion cell layer; IPL, inner plexiform layer; INL, inner nuclear layer; ONL, outer nuclear layer; and OS, outer segments. The latter two layers represent primarily the photoreceptors and were the ones in which the effects of the heat and light treatments could be best visualized as changes in layer thickness. Arrows in panels b, c, d, and h indicate ONL. (**B**) Quantitative documentation of the changes in photoreceptor sensitivity to light damage as a function of the interval between hyperthermia and light exposure. The thickness of the ONL relates directly to the number of surviving photoreceptors (*15, 16*). To measure ONL thickness, retinas were collected from rats treated as described in (A) and processed for light microscopy in an identical manner. Measurements of the ONL in each retina were made at regularly spaced sites ranging from the superior to the inferior retina, as described by O'Steen *et al.* (*19*). Each bar represents the mean (±SEM) of four rats. The data were analyzed by two-way analysis of variance, and significant differences between groups were determined by the Newman-Keuls test. $*0.05 > P > 0.01$; $**P < 0.01$ compared to control.

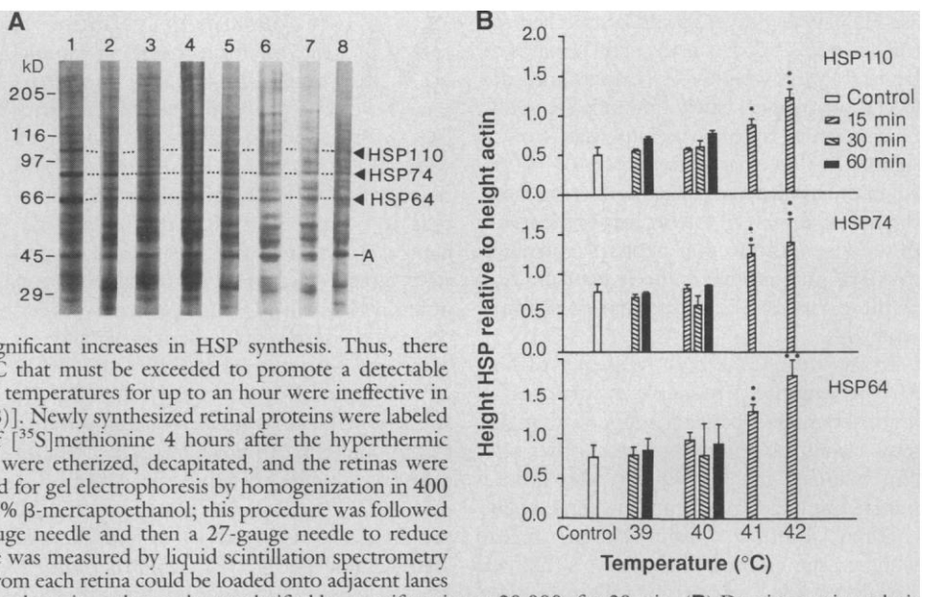

Fig. 2. (**A**) Examples of the effect of different levels and periods of hyperthermia on retinal protein synthesis, shown by fluorography (*20*) of samples of [^{35}S]methionine-labeled retinas analyzed on a 6 to 19% polyacrylamide gradient SDS gel (*21*). The hyperthermic treatments in each lane were: 1, 42°C for 15 min; 2, 41°C for 15 min; 3, 40°C for 60 min; 4, 40°C for 30 min; 5, 40°C for 15 min; 6, 39°C for 60 min; and 7, 39°C for 30 min. Lane 8 is a normothermic control animal. The positions of the three major HSPs and their corresponding approximate molecular size in kilodaltons are indicated on the right, and actin (A) is indicated also to serve as a reference point. Only the two highest levels of hyperthermia caused significant increases in HSP synthesis. Thus, there appeared to be a threshold between 40° and 42°C that must be exceeded to promote a detectable increase in HSP synthesis. Even exposures to lower temperatures for up to an hour were ineffective in triggering the response [densitometric analysis in (B)]. Newly synthesized retinal proteins were labeled in vivo by an intravitreal injection of 200 μCi of [^{35}S]methionine 4 hours after the hyperthermic treatment. Two hours after the injection, the rats were etherized, decapitated, and the retinas were collected by extrusion (*10*). Each retina was prepared for gel electrophoresis by homogenization in 400 μl of a solution containing 2% SDS, 8*M* urea, and 2% β-mercaptoethanol; this procedure was followed by trituration several times, first through a 21-gauge needle and then a 27-gauge needle to reduce viscosity. Total labeled protein in each homogenate was measured by liquid scintillation spectrometry (*22*) so that equivalent amounts of labeled protein from each retina could be loaded onto adjacent lanes of the polyacrylamide gel. Immediately before electrophoresis, each sample was clarified by centrifugation at 20,000*g* for 30 min. (**B**) Densitometric analysis of retinal HSP synthesis in retinal samples from rats treated as described in (A). All three of the retinal HSPs were synthesized at significantly elevated levels only after a rise in body temperature to 41° or 42°C for 15 min. Lower temperatures and longer exposure periods were ineffective. To control for interretinal variation in the absolute amount of [^{35}S]methionine incorporated into newly synthesized retinal proteins, the density of each HSP band (its peak height in the densitometric scan) was divided by the density of the actin band in the same sample. The means and SEMs of these ratios of each HSP band density to the actin band density are represented in the graph. The use of actin as an internal standard for [^{35}S]methionine incorporation is justified because the synthesis of labeled actin was not altered by the hyperthermia (*10*). The number of animals (pairs of retinas) analyzed for each condition were: Control, 4; 39°C for 30 min, 4; 39°C for 60 min, 5; 40°C for 15 min, 4; 40°C for 30 min, 4; 40°C for 60 min, 4; 41°C for 15 min, 7; and 42°C for 15 min, 8. Statistical analysis was done as described in (A). $*0.05 > P > 0.01$; $**P < 0.01$ compared to control.

made it possible to determine if a brief period of hyperthermia could reduce or prevent light-induced photoreceptor cell loss, while at the same time elevating HSP production. The body temperatures of rats were raised to 41°C for 15 min, and then the animals were exposed to bright light at increasing time intervals after the hyperthermia. Two weeks after the light exposure, when all the irreparably damaged photoreceptors were cleared from the retina (*16*), the eyes were removed and processed for light microscopy. Examination of the outer nuclear layer of the photoreceptors in each pair of eyes showed that they were significantly protected against damage when the hyperthermia preceded the light exposure by 10 to 24 hours (Fig. 1A). At 18 hours after hyperthermia, the retinas of the rats exposed to the bright light were completely protected from damage, being statistically indistinguishable from those of the control rats (Fig. 1, A and B). By 50 hours after hyperthermia, however, the protection had waned significantly (Fig. 1, A and B).

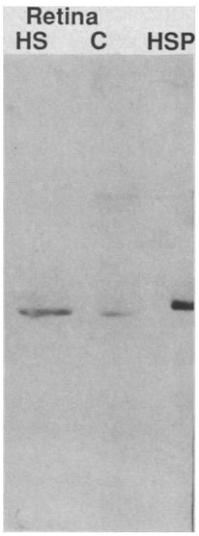

Fig. 3. A representative protein immunoblot of heat-stressed (HS) and control (C) retinal homogenates, and purified HSP72 (HSP) from HeLa cells (*23*) reacted with a monoclonal antibody specific for the stress-inducible form of HSP70. This blot confirmed that HSP64 was immunologically identical to the HeLa cell inducible HSP72 described by Welch *et al.* (*24*). The slight difference in apparent electrophoretic mobility may reflect minor interspecies variation in amino acid composition. In addition, under the conditions used here, HSP72 ran with an apparent molecular size of about 66 to 68 kD; this increase in electrophoretic mobility is probably a result of the increased unfolding of the protein caused by the inclusion of 8*M* urea in the electrophoresis sample solution (*25*). By the same reasoning, we suspect that the retinal HSP74 is identical to HSP83-90 (*24, 26*). Homogenates of retinal proteins and the purified HeLa HSP72 were prepared and analyzed by gel electrophoresis as described in Fig. 2A. The proteins in the gel were transferred to nitrocellulose (*27*), and the blots were incubated with the monoclonal immunoglobulin G (IgG) affinity-purified antibody to HSP72 [C92F3A-5, diluted 1:500 with 2% bovine serum albumin in phosphate-buffered saline; antibody was characterized by Welch and Suhan (*23*)] overnight at 4°C, and then washed in TTBS (0.05% Tween-20 in 50 m*M* tris-buffered saline, *p*H 7.4). A biotinylated rat-adsorbed goat antibody to mouse IgG was used as the secondary antibody. Bound antibody was detected by using an avidin-biotin alkaline phosphatase or horseradish peroxidase detection system (Vectastain ABC-AP or ABC-HRP; Vector Laboratories). Controls included substituting horse serum or an unrelated IgG for the primary antibody and were always negative.

If the greater resistance of the retina to damage is related to the HSPs, then the synthesis of those proteins should be increased and they should accumulate in the retina. To examine the effect of hyperthermia on protein synthesis, the retinas were labeled in vivo by intraocular injections of [^{35}S]methionine 4 hours after a range of hyperthermic treatments and analyzed by one-dimensional gel electrophoresis. An example of the fluorographs of the gels shown in Fig. 2A (*20–22*) illustrates that the synthesis of the three HSPs increased in the animals heated to 41° or 42°C for 15 min. Densitometric analyses of those and replicate fluorographs confirmed that 41°C for 15 min was the threshold temperature treatment needed to elicit significant increases in the synthesis of all three of the previously described (*10*) retinal HSPs (Fig. 2B). Body temperature increases below that, even for up to 60 min, failed to produce a detectable rise in the synthesis of any of the HSPs.

In another set of animals, the accumulation of total HSP64 in the retina 24 hours after hyperthermia was analyzed by using protein immunoblots of polyacrylamide gels of retinal homogenates. The blots were probed with a monoclonal antibody to the 72-kD inducible HSP derived from HeLa cells (HSP72) (*23*). In addition, a sample of the purified HeLa cell HSP72 was run on the same gel to serve as a positive control for the immunodetection procedure. Only the 64-kD retinal protein was recognized by the antibody, and it had a similar electrophoretic mobility as the purified HSP72 (Fig. 3) (*24–27*). Furthermore, the amount of immunoreactive protein in the retina of heat-stressed rat was greater than that in the control, illustrating that hyperthermia did increase retinal content of HSP. Densitometric analyses of a series of similar protein immunoblots of retinas collected at various times after hyperthermia are summarized in Fig. 4. These results indicate that retinal HSP content was maximal 18 hours after the hyperthermic stress, the same time at which maximal protection against light damage was observed.

Our results show that a brief period of hyperthermia in the whole animal alters the cells of the retina so that they are more resistant to subsequent damage. The time after the hyperthermia for the cells to become maximally resistant to stress is consistent with the hypothesis that the cells are synthesizing and accumulating some new proteins that help to prevent the subsequent damage. The most prominent change in protein synthesis that is occurring during the period after hyperthermia is the increase in HSP synthesis. When considered in conjunction with other observations in cultured cells showing a strong correlation between HSP synthesis and subsequent stress tolerance (*4–7*), this result provides further support for the hypothesis that it is the elevated production of the HSPs that makes the retinal cells more resistant to the light-induced damage. Since the retina represents an extension of the CNS, we suggest that our results linking HSP synthesis and stress tolerance may be generally applicable to the entire CNS. Furthermore, HSPs produced by glia at a site of stress can be transferred directly to axons (*28*), thereby providing a route of rapid delivery of these proteins to parts of a neuron distant from the protein-synthesizing soma. Thus, by enhancing the production of HSPs in the nervous system, it may be possible to reduce the irreplaceable loss of nerve cells and their dendrites and axons that occurs after physical injury, disease, or exposure to toxins.

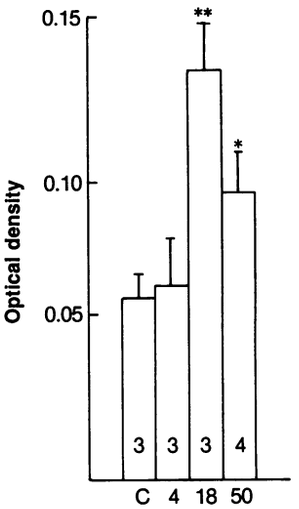

Fig. 4. Densitometric analysis of immunoblots of retinas showing that the rise and fall of total retinal HSP64 with time after hyperthermia parallels the rise and fall of protection of the retina from light damage (Fig. 1). Pairs of retinas were collected from control rats (C) and from those subjected to heat stress to 42°C for 15 min at 4, 18, or 50 hours before collection. Retinas were processed as described in Fig. 2A. The number of rats per group is shown within the bottom of each bar, and each retinal sample was analyzed in triplicate. The data for each pair of eyes per rat were combined to yield a mean value for each rat and those means were used to calculate the SEMs shown in the graph. The group means were compared by a one-way analysis of variance and individual differences were detected by the least squares difference test. *$P < 0.05$ compared to all other groups; **$P < 0.01$ compared to control and to 4 hours after heat stress group. Optical density expressed in arbitrary units.

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Human IL-3 and GM-CSF Act Synergistically in Stimulating Hematopoiesis in Primates

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Interleukin-3 (IL-3) is a member of a family of growth factors, each of which supports the proliferation and development of hematopoietic precursors in culture. Although the biologic effects of the different hematopoietic growth factors have been well documented in different culture systems, it has only recently become possible to study the activities of these molecules in vivo. In comparison with the later acting hematopoietic growth factors granulocyte-macrophage colony-stimulating factor (GM-CSF) and granulocyte colony-stimulating factor, IL-3 elicited a delayed and relatively modest leukocytosis when continuously infused intravenously in primates. The IL-3 infusion, however, greatly potentiated the responsiveness of the animal to subsequent administration of a low dose of GM-CSF. These results suggest that IL-3 expands an early cell population in vivo that subsequently requires the action of a later acting factor such as GM-CSF to complete its development. Optimal stimulation of hematopoiesis may be achieved with combinations of hematopoietic growth factors.

INTERLEUKIN-3 (IL-3) (*1*), ALSO known as multilineage colony-stimulating factor (multi-CSF), is a member of a complex network of interactive cytokines that play an important role in regulating the hematopoietic and immune systems (*2*). Previous studies have shown that IL-3 interacts with early progenitor cells common to most if not all of the myeloid cell lineages (*3–7*). We, as well as others, have reported on the effects of GM-CSF (*8, 9*) and granulocyte colony-stimulating factor (G-CSF) (*10*) in nonhuman primates. We now report the effects of IL-3 alone and in combination with GM-CSF on hematopoiesis.

Continuous intravenous infusion of recombinant human IL-3 expressed either in mammalian cells or bacteria, at a rate of 20 $\mu g\ kg^{-1}\ day^{-1}$, elicited a modest and delayed leukocytosis in normal macaques (Fig. 1). Typically, the white cell counts increased gradually during the 7-day infusion from a mean baseline of 7,750 cells per microliter (range 6,000 to 8,000) to a mean maximum of 17,000 (range 12,000 to 26,000), 1 to 3 days after termination of the treatment in four animals. The increase in leukocyte count was predominantly due to increased levels of neutrophils, eosinophils, and lymphocytes, and an unusual population of leukocytes containing toluidine blue–staining granules (Fig. 2). Although toluidine blue staining is characteristic of normal basophilic granulocytes, the morphology of these cells is atypical in that they are hypogranulated and have a more diffuse chroma-

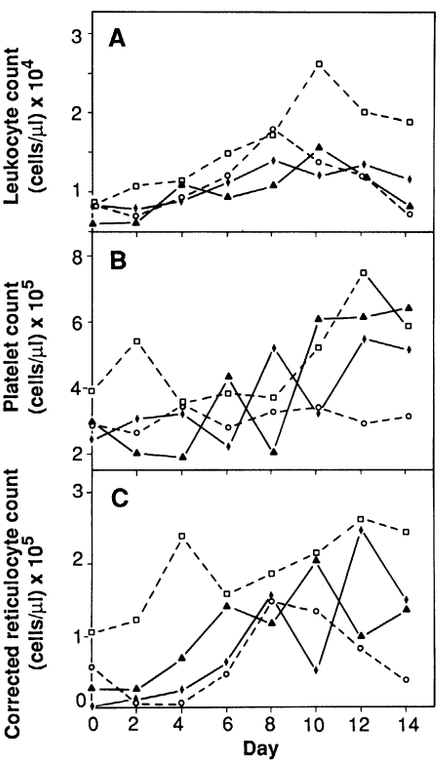

Fig. 1. Effects of continuous infusion of human IL-3 on the levels of circulating blood cells in the cynomolgus macaque (*Macaca fasicularis*). The IL-3 was continuously infused intravenously for 7 days in four different animals (*21*). Daily blood samples were drawn for complete and differential blood cell count analysis. The results from the sample drawn immediately before the infusion was started are indicated as day 0. The data represent the actual values for (**A**) complete white blood cell, (**B**) platelet, and (**C**) corrected reticulocyte counts for four different animals, each infused with IL-3 at the rate of 20 $\mu g\ kg^{-1}\ day^{-1}$.

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