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Antibody Formation: Reduced Responses After Administration of Excessive Amounts of Nonspecific Stimulators

(poly(A·U)/theophylline/mouse/*in vivo*/tissue culture/cyclic AMP)

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ABSTRACT Enhancement of antibody formation *in vitro* and *in vivo* by poly(A·U), cyclic AMP, dibutyryl cyclic AMP, or any one of these agents in combination with theophylline or caffeine, is usually limited to certain dose ranges. High doses of these nonspecific stimulators can produce less or no enhancement, or may actually inhibit antibody formation. Such dose–response relationships may be pertinent to an understanding of phenomena of specific immunological nonresponsiveness and certain types of antigenic competition.

Despite our rapid growth in understanding of the cells involved in the immune response (1–3), there remains uncertainty about the molecular nature of events responsible for the ability of immunogens to induce either a productive activation of immunocompetent cells (including cell-mediated immunity, memory, antibody formation) or a lasting specific nonresponsiveness (tolerance). It is known that tolerance results after exposure of immunocompetent cells to too little or too much antigen, and it is clear that the induction of such "low-zone" and "high-zone" tolerance involves the same cells that can participate in antibody formation when the antigen is administered in appropriate doses (4, 5). Furthermore, recent data (6) indicate that low-zone tolerance affects thymus-dependent (T) cells, whereas high-zone tolerance affects principally bone-marrow-derived (B) cells. Some investigators have speculated that the production of nonresponsiveness may be due to differences in presentation of the immunizing antigen; others have suggested that the lymphocytes involved may be able to distinguish either qualitatively or quantitatively different signals (7–9). The possibility that naturally occurring, cell-released *nonspecific* factors, not antibody in nature, may contribute to immunological nonresponsiveness has received scant attention; however, it has been noted *in vitro* that certain nonspecific activators of lymphocytes, namely phytohemagglutinin and isopentenyladenosine, can produce dose-dependent effects in which the cellular response decreases sharply at too high concentrations of these agents (10, 11). We have now observed that agents that mimic the effects of naturally occurring *nonspecific* stimulators of cells involved in antibody formation (12) may either enhance a specific immune response or inhibit it, the direction of the modified response being a function of the concentration of the modifier.

RESULTS

The synthetic polynucleotide poly(A·U), a known enhancer of humoral and cell-mediated immune responses (13–15), was added in different concentrations to cultures of mouse spleen cells (16) that contained sheep erythrocytes as antigen. Fig. 1 shows that concentrations of 0.1 or 1 ng/ml of poly(A·U) increase the number of cells that form antibodies 3 days later, but less stimulation results at higher concentrations of poly(A·U); a concentration of 100 ng/ml actually inhibits the response. As judged by the frequency of occurrence of dye-excluding cells, there is no cytotoxicity at the inhibitory concentrations, and cultures assayed at later days show no delayed increase in antibody-forming cells.

The exixtence of somewhat similar dose–response relationships *in vivo* was indicated by the previous finding (14) that poly(A·U) administered (*in vivo*) with an antigen produces an optimal degree of stimulation of antibody responses at dosages of about 300 μg per mouse (20-g mouse; administered intravenously); higher doses failed to increase this enhancement, and frequently produced less enhancement. More recent studies (17) revealed that the effects of poly(A·U) *in vivo* and *in vitro* can be significantly potentiated by the concurrent administration of theophylline or caffeine, an effect that is most pronounced when the poly(A·U) concentration

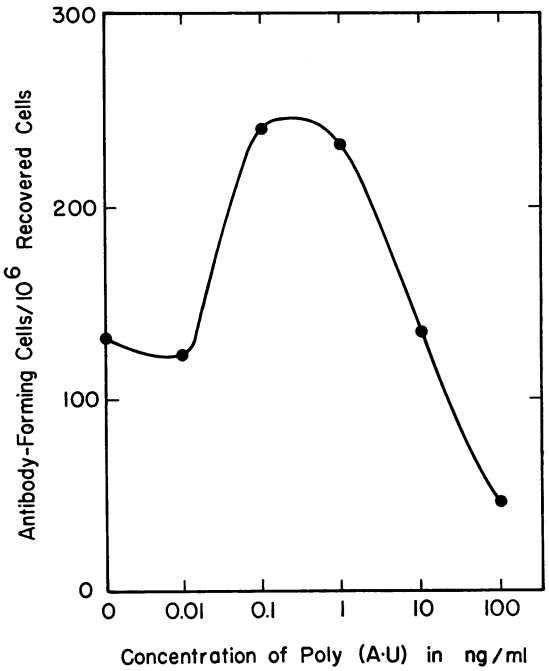

FIG. 1. Effects of different concentrations of poly(A·U) on antibody formation to sheep erythrocytes *in vitro*. The results represent averages of triplicate cultures, initiated with spleen-cell suspensions of CFW mice, assayed on day 3.

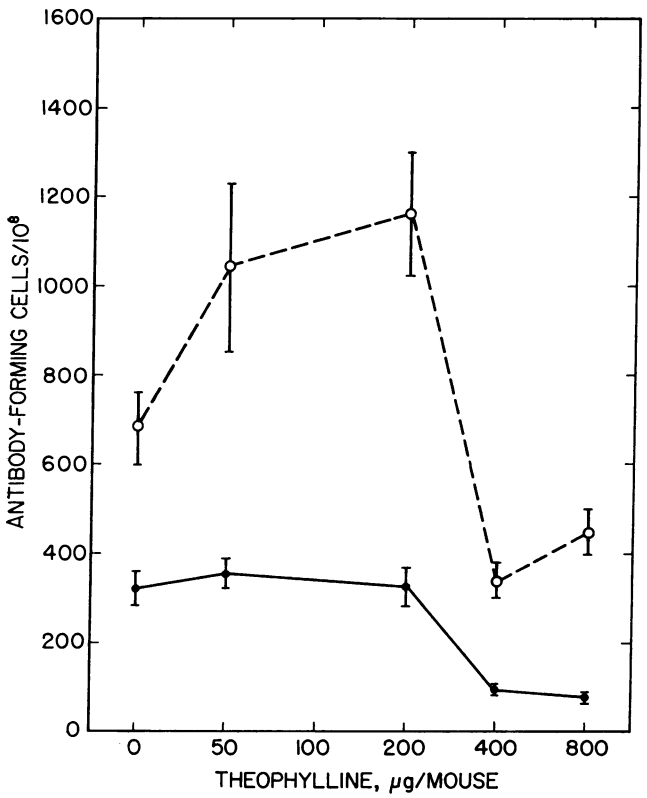

FIG. 2. Effects of different concentrations of theophylline (administered interperitoneally), administered with poly(A·U) (intravenous) at the time of immunization of CFW mice with 10^8 sheep erythrocytes (intravenous), on the number of antibody-forming spleen cells determined 48 hr later. Each point represents the average (±SE) for five mice per group. Note the reduced response after high doses of theophylline alone; this reduction was not accompanied by any detectable toxic effects. The mice weighed 20 g. ●—●, theophylline alone; O- - -O poly(A·U) [30 μg/mouse] plus theophylline.

is kept at a suboptimal dosage, e.g., 30 μg/mouse. Under these conditions, as illustrated in Fig. 2, optimal stimulation of antibody formation is obtained at 200 μg of theophylline; higher concentrations produce less or no stimulation above the extent of response induced by antigen alone.

There is good evidence for the belief that poly(A·U) and theophylline affect the same critical intermediate in the activation of immunocompetent cells, namely cyclic AMP (17). Direct tests for adenylate cyclase activities in spleen cells (18) have shown that poly(A·U) is an enhancer of the enzyme responsible for the conversion of ATP into cyclic AMP, and it is well established that both theophylline and caffeine, by inhibiting phosphodiesterase activity, prevent the rapid conversion of cyclic AMP into biologically inactive AMP (17). Thus, the combined effects of poly(A·U) and theophylline may be attributed to an elevation of, and longer persistence of, cyclic AMP concentrations in immunocompetent cells that have been activated by antigen. It appears that whenever such alterations of cyclic AMP concentration exceed a certain level, responses decline rather than increase.

The role of cyclic AMP-mediated events in immune responses has been substantiated by the demonstration that cyclic AMP itself, as well as dibutyryl cyclic AMP, administered with antigen, can stimulate antibody formation *in vivo* (17) and *in vitro* (19, 20). Again, such stimulatory effects, which are presented in more detail elsewhere (18–20), are limited to certain dose ranges, with higher concentrations resulting in less or no stimulation and sometimes even an inhibited response (Table 1). Note also in the examples of Table 1 that theophylline, at a concentration that is known to potentiate the effects of *suboptimal* stimulatory concentrations of poly (A·U), cyclic AMP, or dibutyryl cyclic AMP, will abolish enhanced responses (and may even cause inhibited responses) when added to doses of the cyclic nucleotides that by themsleves cause near-*optimal* enhancement. Similarly, adenylate cyclase activity of spleen cells is increased after *in vitro* exposure to phytohemagglutinin (1 μg/ml) or to ApApA (1 μg/ml), but is decreased after exposure of the cells to a combination of these stimulatory agents (18–20).

TABLE 1. *Dosage-dependent effects of dibutyryl cyclic AMP and cyclic AMP on antibody formation in vivo and in vitro*

Treatment of spleen donors or spleen cell cultures	Average number of antibody-forming cells 48 hr after immunization (*A*) or 72 hr after start of spleen cell cultures (*B*)
A. In vivo	
Unimmunized controls	26.3 ± 3.4*
10^8 sheep erythrocytes	448.8 ± 28.4
10^8 sheep erythrocytes + 500 μg dibutyryl cyclic AMP	1003.5 ± 98.2
10^8 sheep erythrocytes + 1000 μg dibutyryl cylic AMP	336.9 ± 21.7
10^8 sheep erythrocytes + 2000 μg dibutyryl cyclic AMP	302.4 ± 20.6
10^8 sheep erythrocytes + 500 μg dibutyryl cyclic AMP + theophylline†	345.8 ± 18.0
B. In vitro	
Antigen-free control	7.5
sheep erythrocytes	148.0
sheep erythrocytes + 0.1 μg/ml cyclic AMP	239.4
sheep erythrocytes + 1 μg/ml cyclic AMP	248.5
sheep erythrocytes + 10 μg/ml cyclic AMP	160.0
sheep erythrocytes + 0.1 μg/ml cyclic AMP + 1 ng/ml theophylline	178.2
sheep erythrocytes + 1 ng/ml theophylline	163.8

In the *in vivo* tests (5 mice [average weight, 20 g] per group) dibutyryl cyclic AMP and/or theophylline, in the form of $(\text{theophylline})_2$ ethylenediamine, were injected at time of immunization. All injections, unless indicated otherwise, were intravenous. In the *in vitro* tests (3 cultures per group), the spleen cells were treated with the drugs for 10 min at 37°C, washed, and then placed in culture.

* Standard error. Similar statistical treatment could not be applied to the *in vitro* data since, like others (16), we pooled spleen cell suspensions for initiating *in vitro* cultures in triplicate and then assayed each culture in duplicate.

† 100 μg subcutaneously at the time of immunization.

DISCUSSION

An obligatory involvement of cell-released nonspecific stimulatory substances that affect T and B cells, particularly the latter, in the initiation of any specific antibody response has long been suspected (12), and has been the object of a number of studies (21–23). In particular, some recent studies have indicated that activated T cells may be the source of an important nonspecific factor that supports the performance of antigen-activated B cells (24 and personal communication by J. Ketterman and R. Dutton). In contrast to these nonspecific *stimulatory* effects, it has also been recognized that there exist *inhibitory* substances that can be produced by one cell type and affect the performance of another cell type involved in antibody formation (25–27). However, the identity of such regulatory substances and their contribution to phenomena of specific nonresponsiveness has remained obscure. The present data suggest that enhancing and inhibitory modifiers of immune responses could represent one and the same material to which cellular responses differ at different concentrations. Similar dosage-dependent effects, where qualitatively different responses are elicited at different concentrations of the same compound, have been noted in studies with hormone-dependent cells (28), which underscores the parallelism of early intracellular events in activated hormone-dependent and antigen-dependent cells (17).

It will be interesting to determine to what extent an excess of nonspecific stimulators, released from endogenous sources after immunization (12, 21–24), might contribute to the nonresponsiveness occurring after the immunization of animals with high doses of antigen; conversely, it will be of interest to ascertain whether a lack of presumably obligatory nonspecific stimulators may contribute to low-dose tolerance after low doses of antigen are administered. Finally, it should be interesting to explore the role of excessive amounts of endogenous nonspecific stimulators in evoking certain phenomena of antigenic competition (29–31).

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