Suppression and Stimulation of the In Vitro Immune Response by Chlorpromazine

Kunihiko Ichimura

Chemistry Division, National Institute of Radiological Sciences, Chiba

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Previous in vivo studies indicated that chlorpromazine (CPZ) caused partial suppression of primary immune response, i.e., a slight delay in the onset of the log phase as well as in the time of the peak response of antibody formation in mice. The suppression appeared to be due to interference of CPZ with an initial proliferative response of antibody-forming cells and their precursors [6]. Several reports have also suggested that the suppressive effect of CPZ may be related to the inhibition of cell proliferation or DNA synthesis [1, 12]. However, the precise nature of its effects remained obscure. Recently, it has been reported that CPZ inhibits an early cellular interacting process in a lymphocyte response to concanavalin A mitogen rather than the subsequent process of DNA synthesis and cell proliferation [4].

In this report, therefore, I have employed in vitro humoral immune systems as a clearer approach to the investigation of the effect of CPZ on immune response. The results reported in this paper show that CPZ, depending on the concentration, had enhancing as well as suppressive effects on direct plaque-forming cell (D-PFC) response in vitro. It was also found that the suppressive action of CPZ, in relatively low concentrations (5 × 10⁻⁶ to 5 × 10⁻⁵ M), mainly affected the initial earliest step during the course of D-PFC response. These findings suggest that the observed immunosuppression might be attributed mainly to the impairment of cellular interacting events preceding the processes of DNA synthesis and cell proliferation.

Chlorpromazine (Takeda Chemical Industries, Ltd., Osaka, Japan) was diluted with physiological saline and used in the concentrations described below. The effect of CPZ on humoral immune response was tested by determining the capacity of mouse spleen cells to produce D-PFC response to sheep erythrocytes (SRBC) in the presence of the drug. The culture method was a modification [9] of the method described by Marbrook [10]. Normal or preimmunized (intraperitoneally with 2 × 10⁸ SRBC 30–60 days previously) C57BL/6J spleen cells (10⁷) with SRBC (10⁷) were added to the inner chambers, and CPZ at various concentrations was added to the outer chambers of the culture vessels. After 4 days of incubation the cultures were assayed for D-PFC by the technique of Jerne et al [8]. The number of viable nucleated cells was determined by the trypan blue exclusion procedure. The culture medium used was Eagle's minimum essential medium (with 60 µg/ml of kanamycin, Nissui Seiyaku Co., Ltd., Tokyo). It was supplemented with sodium pyruvate, nonessential amino acids [9], and 5% (for use in the secondary immune system) or 10% (for use in the primary immune system) fetal calf serum (Grand Island Biological Co., Grand Island, N.Y., U.S.A.). Under these culture conditions, positive control cultures of the primary and secondary immune systems showed a similar characteristic D-PFC response profile with peak numbers of 606 ± 76 (mean ± SEM) and 762 ± 97 per 10⁶ recovered cells on day 4 after the addition of antigen, respectively. In contrast, no indirect (IgG) PFC response was detected for 4 days in these culture systems.

The effect of CPZ on primary and secondary D-PFC response is shown in Figure 1. When CPZ was present in cultures during the incubation period, CPZ concentrations of more than 5–7.5 × 10⁻⁶ M had a complete inhibitory effect on the D-PFC responses. On the other hand, concentrations between
5 × 10⁻⁷ and 5 × 10⁻⁶ M had a slightly stimulative effect and concentrations of less than 5 × 10⁻⁷ M had little or no effect. A striking similarity in the CPZ dose-response curves of both systems was found although the minimum inhibitory concentration was shifted slightly to the right in the primary D-PFC response system. This shift, however, was found to be due merely to the differences in the concentration of fetal calf serum in the culture media (data not shown). Therefore, there seems to be no essential differences in susceptibility to CPZ between primary and secondary D-PFC response.

Subsequent experiments were designed to test whether or not there is a sensitive phase to the drug during the induction of antibody response. An in vitro secondary immune system was used for these experiments. Therefore CPZ was added at various times during the incubation period. The cultures were exposed to CPZ at 1.2–1.5 × 10⁻⁵ M for 12 or 24 hr. The cytotoxicity of this drug was tested by the trypan blue exclusion assay. The viable cell recoveries of the treated groups did not differ significantly from those of the untreated control groups (Table 1). This means that CPZ in these concentrations was not detectably cytotoxic to lymphocytes.

As shown in Figure 2, most of the decrease in D-PFC response produced by CPZ occurred when the drug was present during the earliest period (0–24 hr) of incubation. In contrast, if CPZ was present during the latest period (72–96 hr), PFC response was slightly stimulated. Further experiments indicated that the most sensitive phase to CPZ was between 0 and 12 hr after antigen stimulation (Fig. 3). It has been well documented

Table 1. The effect of CPZ on cell recovery at various times of culture

<table>
<thead>
<tr>
<th>Expt.</th>
<th>Hours of culture</th>
<th>Cultures</th>
<th>Control</th>
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<tr>
<td></td>
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<td>Exposed to CPZ(a)</td>
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<td>(1.5 × 10⁻⁵ M, for 12 hr)</td>
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<td></td>
<td>12</td>
<td>51.5 ± 3.7</td>
<td>57.3 ± 1.3</td>
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<td></td>
<td>24</td>
<td>43.5 ± 1.9</td>
<td>43.6 ± 1.6</td>
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<td></td>
<td>36</td>
<td>38.1 ± 0.6</td>
<td>38.7 ± 1.2</td>
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<td>48</td>
<td>27.8 ± 0.3</td>
<td>29.3 ± 1.6</td>
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<td>(1.5 × 10⁻⁵ M, for 24 hr)</td>
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<td></td>
<td>24</td>
<td>38.1 ± 1.8</td>
<td>45.7 ± 1.2</td>
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<td></td>
<td>48</td>
<td>28.7 ± 1.7</td>
<td>31.8 ± 1.2</td>
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<td></td>
<td>72</td>
<td>26.1 ± 1.1</td>
<td>25.1 ± 0.6</td>
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<tr>
<td></td>
<td>96</td>
<td>26.1 ± 2.0</td>
<td>25.9 ± 1.0</td>
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(a) The cell recovery was expressed as per cent of cells present initially.
(b) SRBC antigen was added to each group of cultures at the start of culture.
(c) Twelve or twenty four hours before assaying the cell recovery, CPZ was added to the cultures. The cell recovery was tested by counting the viable cells harvested at various culture hours indicated.
(d) Mean of three experiments, including triplicate cultures, ± the standard error of the mean.
that there is a lag period of 24 to 36 hr after antigenic stimulation in the culture, and after this lag, a phase follows in which DNA synthesis and cell division occurs [2, 11]. Therefore, the results of the pulse experiments in this paper suggest that the immunosuppressive effect of CPZ is related mainly to the impairment of the initial early cellular events in the sequences of immune response rather than the subsequent DNA synthesis and cell proliferation. Thus, it seems that the inhibition of initial cellular events leads to the impairment of subsequent DNA synthesis and cell division, which further results in a decrease in D-PFC response. On the other hand, if CPZ was added at the time when active DNA synthesis and cell division were expected to occur (between 24 to 72 hr after antigenic stimulation), 12-hr exposure had little or no suppressive effect (Fig. 3). However, prolonged exposure, i.e., 24-hr exposure, was found to cause partial suppression of the D-PFC response (Fig. 2). Whether or not the partial suppression is caused by direct suppression of DNA synthesis and cell proliferation remains to be determined. However, other experimental systems and methods are required to examine these possibilities.

The mechanism of the impairment of an early step in this system is unknown. However, other investigators [3] indicated recently that CPZ inhibited mitogen-induced lymphocyte aggregation or clustering and it eliminated the opportunity for cell-cell interaction in the early phase of response. In addition, CPZ dose-response inhibition curves observed in this paper were very similar to those reported by these other investigators. On the other hand, the mechanism by which CPZ at a restricted low concentration ($5 \times 10^{-7}$ to $5 \times 10^{-6}$ M) stimu-
lates D-PFC response (Fig. 1) is also a subject of speculation. Considerable data have indicated that CPZ affects lymphocyte membrane movements or activities, such as cap formation [13], phagocytosis [6], modulation of the cyclic nucleotide level [4] and calcium ion flux [5, 7]. Therefore, it is possible that CPZ at low concentrations may enhance these membrane functions or movements which might form an essential part of the lymphocyte-triggering mechanism. This possibility appears to be supported by the previous finding [6] that CPZ at relatively low concentrations enhanced in vivo erythropagocytosis of reticuloendothelial cells in spleens and livers of mice although it inhibited this function at higher concentrations. However, this possibility must be substantiated by other experimental means in vitro.

In contrast, another concept is needed to explain the mechanism of CPZ-enhanced D-PFC response (Fig. 2) when CPZ was added at very late (72-96 hr after antigenic stimulation), since CPZ at these concentrations was inhibitory to lymphocyte surface movements and cell-cell interactions. Further studies on these phenomena are now in progress.

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REFERENCES


