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# The preferential effects of chloroquine on the IgM and IgG subclass responses to TI and TD antigens in BALB/cAn mice

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**Abstract**—This study has determined the effects of chloroquine on the IgM and the IgG subclass responses of BALB/cAn mice to both thymus-independent (TI) and thymus-dependent (TD) antigens, and has found that in antigen-unprimed BALB/cAn mice chloroquine adversely affects antibody responses to both TI and TD antigens. However, in antigen-primed mice the immune responses to TD antigens were unaffected by chloroquine. The IgG1 subclass response, but not necessarily other IgG subclass or IgM responses to TI and TD antigens, was adversely affected by chloroquine in unprimed mice only. Thus, in unprimed, but not in antigen-primed, BALB/cAn mice, chloroquine preferentially decreased the IgG1 subclass responses to both TI and TD antigens.

Chloroquine and related compounds are anti-inflammatory agents that affect a variety of immune system activities including lymphocyte activation (Bygbjerg & Flachs 1986), delayed-type hypersensitivity reactions (Thong & Ferrante 1980), and antibody production (Greenwood 1984). The effects of chloroquine on some activities of immune systems, antibody production in particular, are variable; for example, the antibody response to yellow fever vaccine is not adversely affected by chloroquine treatment (Tsai et al 1986), whereas the antibody responses to both rabies vaccine and sheep red blood cells in chloroquine-treated individuals are suppressed (Taylor et al 1984; Bhattacharya et al 1984). Why such variant effects of chloroquine on antibody formation occur, whether due to dosage, administration protocol, or to certain aspects of antigen-specific immune responses is not known.

Most antigens can preferentially stimulate IgG subclasses; for example, the TD antigen trinitrophenyl (TNP)-bovine serum albumin (BSA) preferentially stimulates IgG1; the TI antigen TNP-lipopolysaccharide (LPS) preferentially stimulates IgG2b and IgG3; and the TI antigen TNP-Ficoll and TD antigen Group A (GA) streptococcal vaccine preferentially stimulate IgG3 (Slack et al 1980). Other modulators of immune system activities including anti-inflammatory agents such as chloroquine may also preferentially affect the production of certain antibody isotypes. Such preferential effects of chloroquine on antibody isotype production could limit the effects of this compound to only those antigens with certain isotype preferences, thus, explaining at least in part the variable effects of chloroquine on antibody production. To test this possiblity, we have examined whether chloroquine can preferentially affect the antigen-specific IgM and IgG subclass responses of BALB/cAn mice to TI and/or TD antigens.

## Materials and methods

Female BALB/cAn mice aged 5 weeks in groups of 4 were injected weekly for 2, 3, or 4 weeks with 400 μg of chloroquine in 0·9% NaCl (saline) intraperitoneally (i.p.). Three days later, chloroquine-pretreated mice were injected i.p. with 100 μg of $TNP_{56}$-Ficoll in saline, 100 μg of TNP-LPS in saline, or GA-vaccine (Slack et al 1980). Also, some chloroquine-pretreated mice were injected with 400 μg of TNP-BSA in complete Freund's adjuvant (CFA) followed 1 month later with 100 μg antigen in incomplete Freund's adjuvant (IFA). In some cases, mice were first injected with the antigen TNP-BSA in CFA followed 1 month later with chloroquine for 2, 3, or 4 weeks; 3 days later these mice were given TNP-BSA in IFA. Five or 7 days following the final administrations of antigen, spleen cells secreting BALB/cAn TNP-specific or GA-carbohydrate-specific antibody of the IgM class and of the IgG1, IgG2a, IgG2b, and IgG3 subclasses were detected by isotype-specific antisera in antigen-specific plaque assay systems (Slack et al 1980).

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## Results and discussion

The abilities of both TI and TD antigens to stimulate IgM and IgG subclass responses in chloroquine-pretreated and untreated BALB/cAn mice were evaluated initially (Fig. 1). The results indicate that the IgG1 responses to all TD antigens, but to only some TI antigens, are particularly sensitive to chloroquine. Compared with the TNP-BSA-induced IgG1 response in untreated mice of 1,205 IgG1 PFC/$10^6$ spleen cells, the anti-TNP-BSA IgG1 PFC responses in mice pretreated with chloroquine for 2, 3, or 4 weeks were reduced by approximately 50, 60 and 65%, respectively. The responses of the remaining IgG subclasses to TNP-BSA in chloroquine-pretreated animals were reduced from 40 to 45%. Similarly, in mice pretreated for at least 4 weeks with chloroquine, the IgG1 responses to both GA-vaccine and TNP-Ficoll were also preferentially reduced. In contrast, the IgG subclass responses to TNP-LPS were not reduced by treatment with chloroquine. Also, in contrast to the sensitivities of IgG subclass responses to chloroquine treatment, the IgM responses to all antigens were insensitive to chloroquine treatment.

One possible explanation for the abilities of chloroquine to cause decreased immune responses to all TD antigens may be an increased sensitivity of TD-antigen-primed mice to chloroquine. To test this possibility, the sensitivities by isotype of the anti-TNP-BSA response to chloroquine in antigen-primed BALB/cAn mice were examined. The results of this experiment (Table 1) indicate that TNP-BSA induced in antigen-primed animals subsequently treated with chloroquine, both IgM and IgG subclass responses similar to those induced in untreated BALB/cAn mice. We can, therefore, conclude that chloroquine reduces IgG subclass responses, particularly IgG1 subclass responses induced by TD and some TI antigens, and that susceptibility to

Table 1. The effects of chloroquine on the TNP-specific IgM and IgG subclass responses in TNP-BSA primed BALB/cAn mice. [a]TNP-BSA/CFA primed mice in groups of four were injected with antigen/IFA after the second, third, or fourth week of chloroquine treatment. Five days following the final administration of antigen, PFC responses were measured. Shown are mean PFC values and standard error (s.e.) factors. The values of IgG2 PFC are sums of IgG2a and IgG2b subclass PFC.

| Chloroquine[a] treatment (weeks) | PFC/$10^6$ Spleen cells (s.e. factors) | | | |
|---|---|---|---|---|
| | IgG1 | IgG2 | IgG3 | IgM |
| 2 | 1,145 (1·2) | 125 (1·3) | 65 (1·2) | 10 (1·4) |
| 3 | 923 (1·1) | 110 (1·2) | 50 (1·1) | 10 (1·2) |
| 4 | 875 (1·2) | 97 (1·1) | 40 (1·2) | 5 (1·1) |

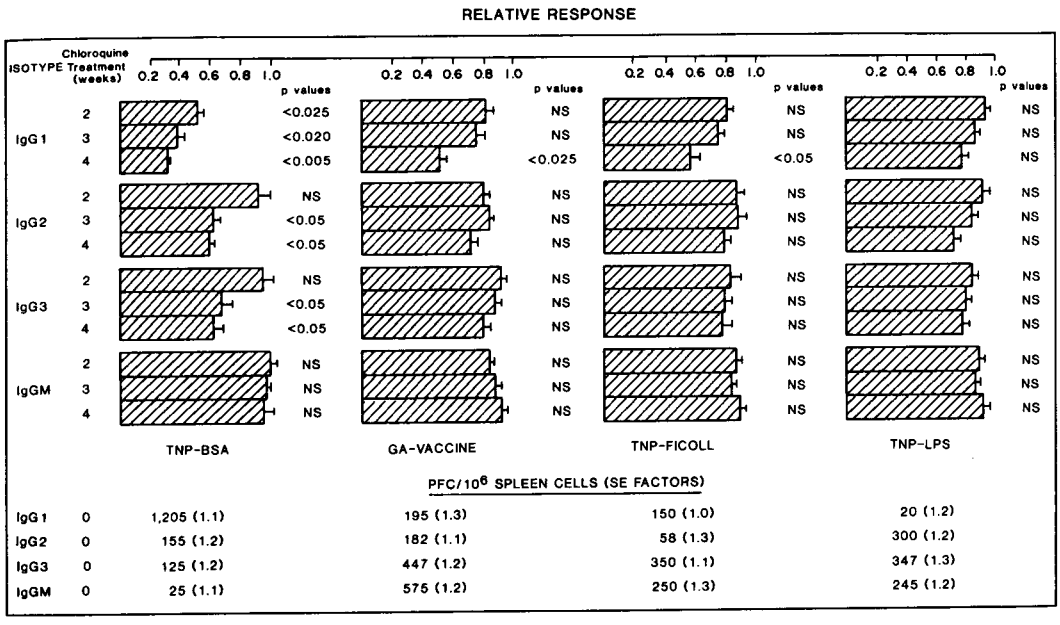


FIG. 1. Mice in groups of four that were either untreated or pretreated for 2, 3, or 4 weeks with chloroquine were immunized with the antigens listed above. On days 5 or 7 following the final antigen administration, IgM and IgG antigen-specific PFC were measured. The PFC responses in untreated mice are shown as PFC/$10^6$ spleens cells and those in treated mice are displayed as response ratios (PFC responses in chloroquine-pretreated BALB/cAn mice relative to those in untreated mice). The statistical significance of differences between mean PFC values in chloroquine-pretreated and untreated mice were evaluated by the Student's *t*-test. The *P* values indicating 95% confidence levels were accepted as significant. The values of IgG2 PFC represent sums of IgG2a and IgG2b subclass PFC.

chloroquine's effect of reducing antibody production resides primarily in unprimed animals.

Our observation that chloroquine can selectively reduce production of a single isotype of antibody in unprimed animals might be important for two reasons. First, it may provide a basis for understanding for some of the varied effects of this agent on antibody production (Greenwood 1984; Taylor et al 1984; Bhattacharya et al 1984; Tsai et al 1986), which might at least in part reflect the ability of chloroquine to affect only certain antigen-induced IgG subclass antibody responses. Second, it may alert clinicians to the potential risks of administering chloroquine to unvaccinated individuals. For example, in individuals receiving chloroquine long-term for malarial prophylaxis (Cohen 1974), a protective TD antibody IgG subclass response to vaccines might be induced effectively in chloroquine-treated, prevaccinated individuals but not necessarily in individuals given chloroquine before vaccination.

How chloroquine could selectively affect production of a single isotype of antibody is unknown. Hypothetically, some of the ability of chloroquine to preferentially affect isotype production might be attributed to the lysosomotropic property of this agent (DeDuve et al 1974). Lysosome-sequestered chloroquine might interfere with cellular activities of immune responses, such as macrophage functions including antigen processing, that may be necessary for the production of T cell-derived cytokines and certain cytokine-dependent isotypes, such as IgG1 (Vitetta et al 1985). This possibility, if it were true, would explain why isotype responses to the TI antigen TNP-LPS are completely insensitive to chloroquine because the mitogenic LPS moiety of this antigen may allow TNP-LPS to bypass the need for macrophage functions in stimulating antibody responses. Therefore, in this manner, and/or possibly in other ways, chloroquine-like antigens and other stimulants of immune systems may be able to preferentially affect the production and function of certain antibody isotypes.

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