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Inhibition of Antibody Formation by Tetracycline on the Cellular Level

Insufficient antibody production caused by therapy of bacterial diseases with commonly used antibiotics, especially chloramphenicol[1] and tetracyclines is explained by suppression of the infectious agent in the macroorganism, *i. e.* by an insufficient antigenic stimulus. We demonstrated that immunosuppression caused by tetracycline is a general immunological problem because this antibiotic during primary and secondary response temporarily diminishes antibody production by a direct effect on antibody producing mammalian cells even after stimulation with antigen which cannot be directly influenced by the antibiotic. We confirmed our former findings in a new series of experiments on the cellular level. The effect on the primary and secondary response *in vivo*, and the secondary response *in vitro* was studied. Results presented in the graphs summarize data of a series of experiments performed and repeated in the course of 2 years.

Materials and methods

Animals. In each experiment 150 five-week-old non-inbred mice weighing about 20 g were divided into two groups.

Antigen. Washed (3×) sheep red blood cells (RBC) were used for primary immunization as a 3 % suspension, for secondary immunization as a 0.5 % suspension 21 days following the first stimulus.

Immunization. 0.1 ml of the respective RBC suspension was injected into each hind foot pad of mice. To ensure maximum effect on antibody-forming cells the antibiotic (Rolitetracycline-Syntetrin, Bristol) was administrated daily by the same route.

Regional lymph nodes were used for the Jerne hemolytic plaque test[2] in Šterzl's modification[3]. Serum was collected from bled mice and 50 % hemolysis was determined photometrically[4,5]. For the identification of IgG antibody producing cells rabbit serum against mouse IgG was prepared[6]. In *in vitro* studies of the secondary immune response purified and concentrated phage T2 was used as antigen. The primary stimulus consisted of two injections of phage T2 (1.2×10^{10} PFU in 0.5 ml and 0.8×10^{10} PFU in 0.5 ml, respectively) applied to rabbits into both hind foot-pads in 2 week intervals. Cultures of 2 mm fragments of regional lymph nodes and of bone marrow were established 6 months after the primary stimulus in Leighton tubes using Parker's medium supplemented with 20 % inactivated bovine serum. The secondary antigenic stimulus was applied *in vitro* and consisted of 10^8 PFU of phage T2 per 1 ml culture medium. Medium was changed in intervals as shown in Fig. 5 and its neutralizing activity was determined. Tetracycline was added to cultures (1 mg per 1 ml medium) at their establishment and at each change of medium. Results were calculated as 100 mg of tissue releasing antibody into 1 ml of medium and was expresed as "K".

Results

A. *The effect of tetracycline on the primary response in vivo.*

Twelve mice of control and TC-treated groups were sacrificed daily for 6 consecutive days. In the course of 3 days the primary response was inhibited by TC the total dose of which amounted to 16 to 17 mg (the first dose was applied 2 hrs after active immunization, and the other two doses on day 2 and 3). Fig. 1 shows that

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inhibition occurs on day 4 after application of antigen when the number of hemolytic plaques from 10^8 lymphoid cells obtained from lymph nodes of the TC-treated group was 30× lower. On day 5, when the production of hemolytic plaques was maximal in the control group, the inhibition in the

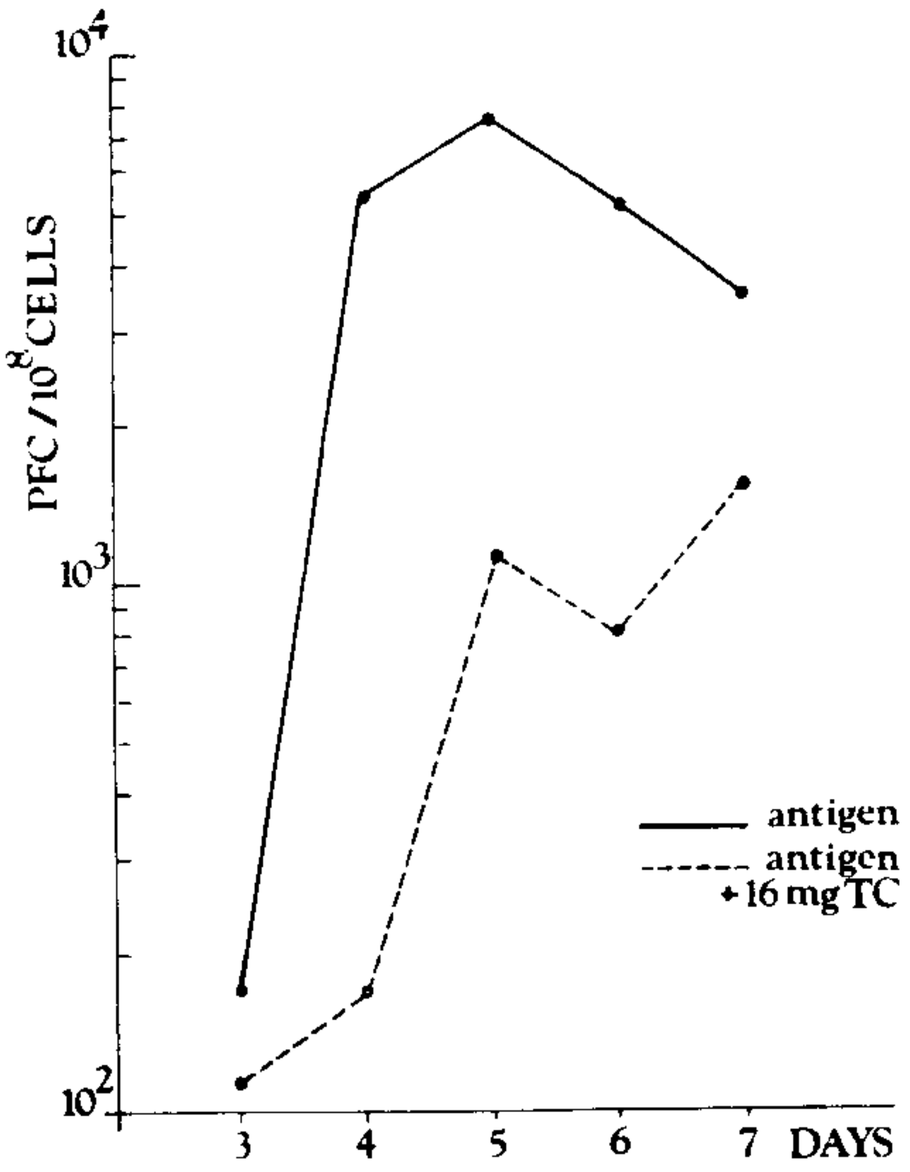

Fig. 1. Primary response.

TC-treated group is still very well marked and remains so till day 6 and 7 when the experiment was terminated. These experiments clearly demonstrate the inhibition of antibody production, especially of IgM by TC.

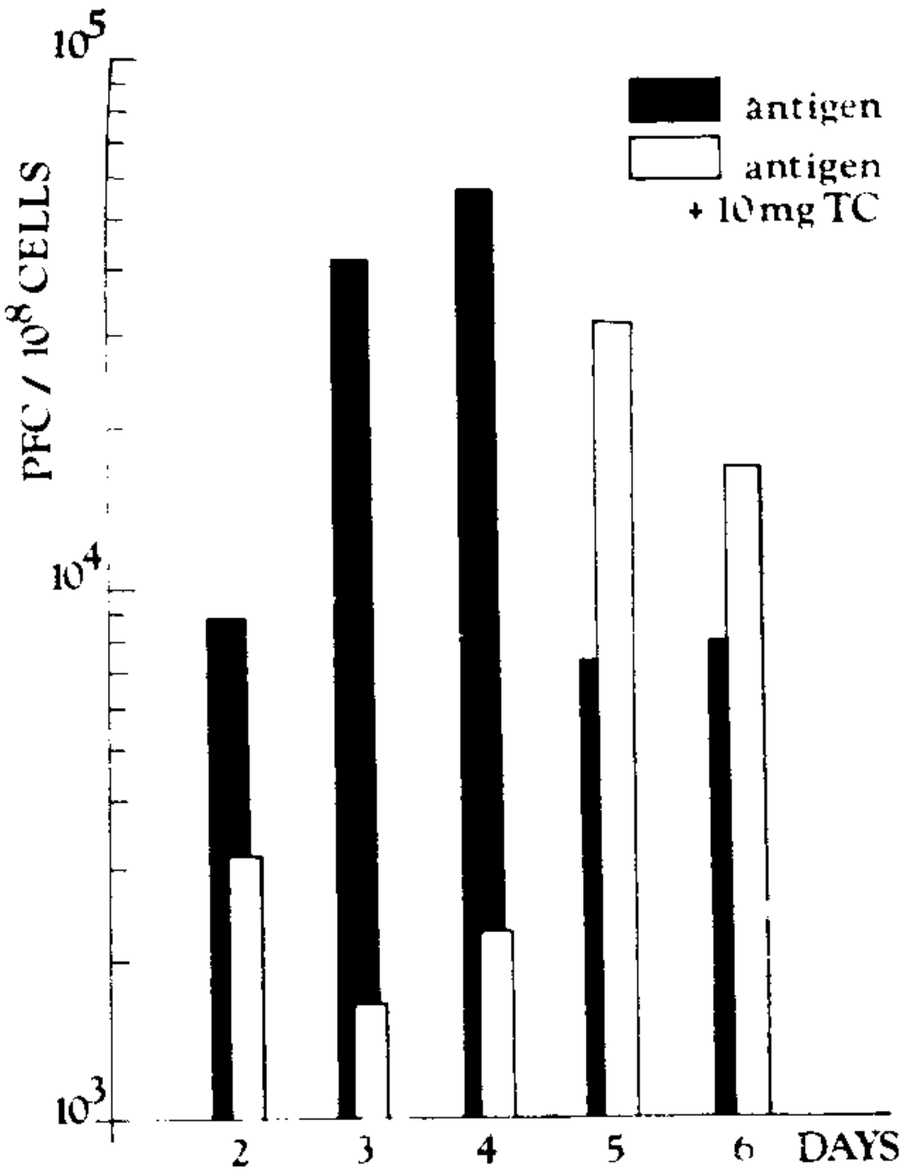

Fig. 2. Secondary response — IgG

B. *The effect of tetracycline on the secondary response in vivo.*

Mice received 10 mg TC in 2 days, the first dose 1 hr after administration of antigen. Cells producing IgM and IgG were differentiated by the direct and indirect plaque test using rabbit antiserum against mouse IgG. Fig. 2 shows that a significant reduction of IgG producing cells occurs on day 2, 3 and 4. On day 3 the production of hemolytic plaques is 24× lower than in the control group. On day 4 the number of plaques in the TC-treated group is 22× lower. Fig. 3 shows that the suppression of IgM producing cells is very weak on day 2 and 3. On day 4 the suppressive effect of TC disappears.

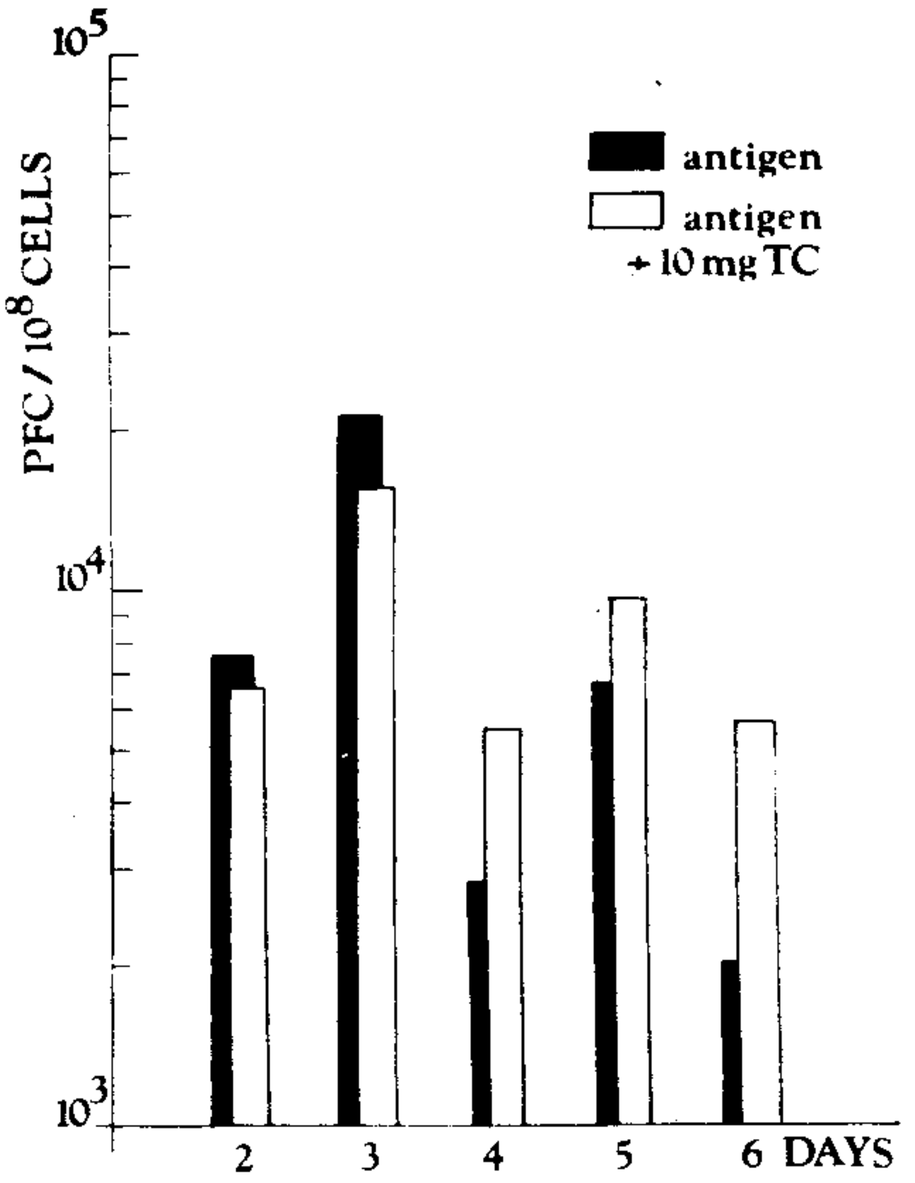

Fig. 3. Secondary response – IgM

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C. *Comparison of dynamics of hemolylic antibody tiler in the secondary response.*

As against control mice in the TC-treated group is apparent a marked inhibition on day 2, 3 and 4 (Fig. 4). On day 5 the titer of hemolytic antibodies approaches the level of the control group and on day 6 the levels are equal.

D. *Suppression of the secondary response in vitro.*

Phage and TC were added to cultures of bone marrow of animals primarily immu-

nized with phage T2. Fig. 5 shows that on the 5th day of cultivation with 1 mg TC a significant suppression of antibody production was attained ($K < 0.01$). An ana-

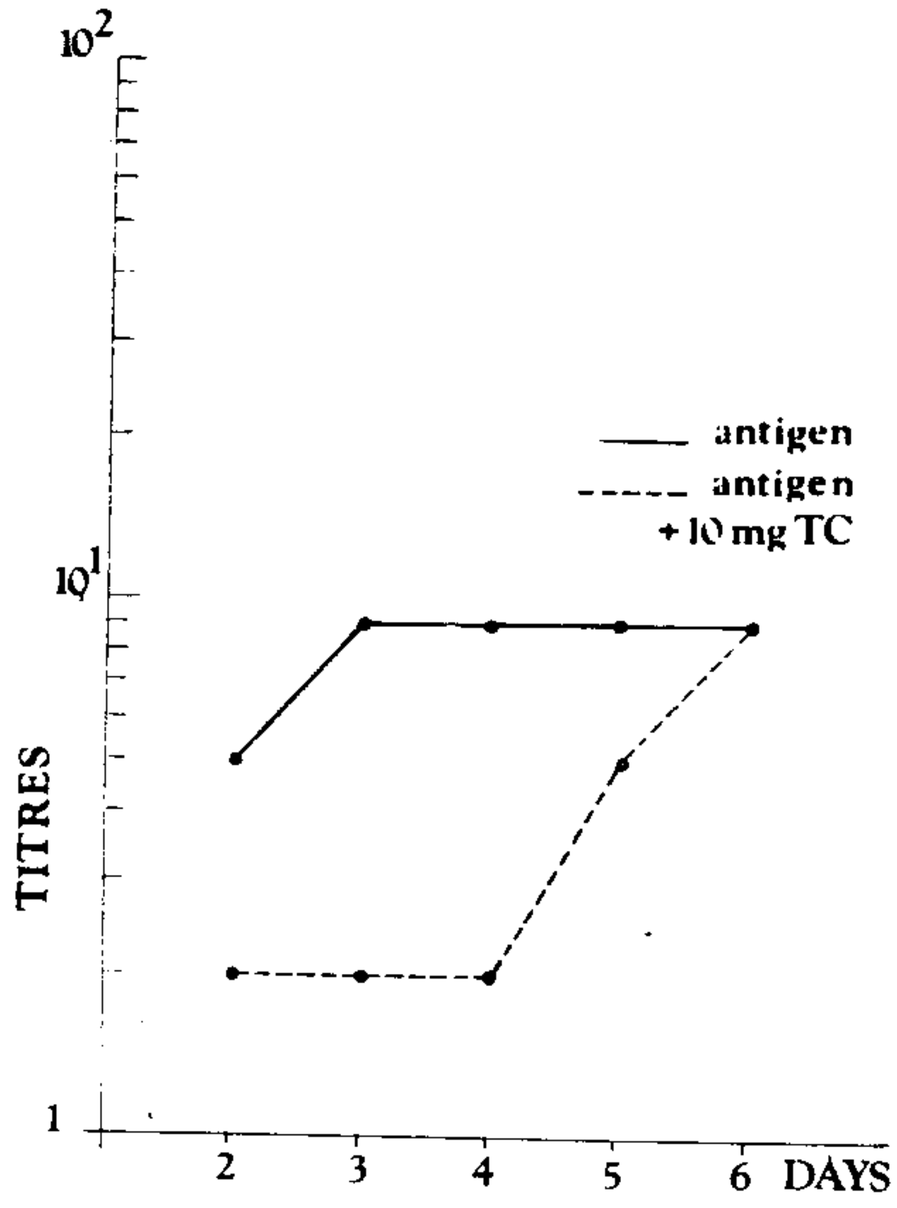

Fig. 4. 50 % hemolysis.

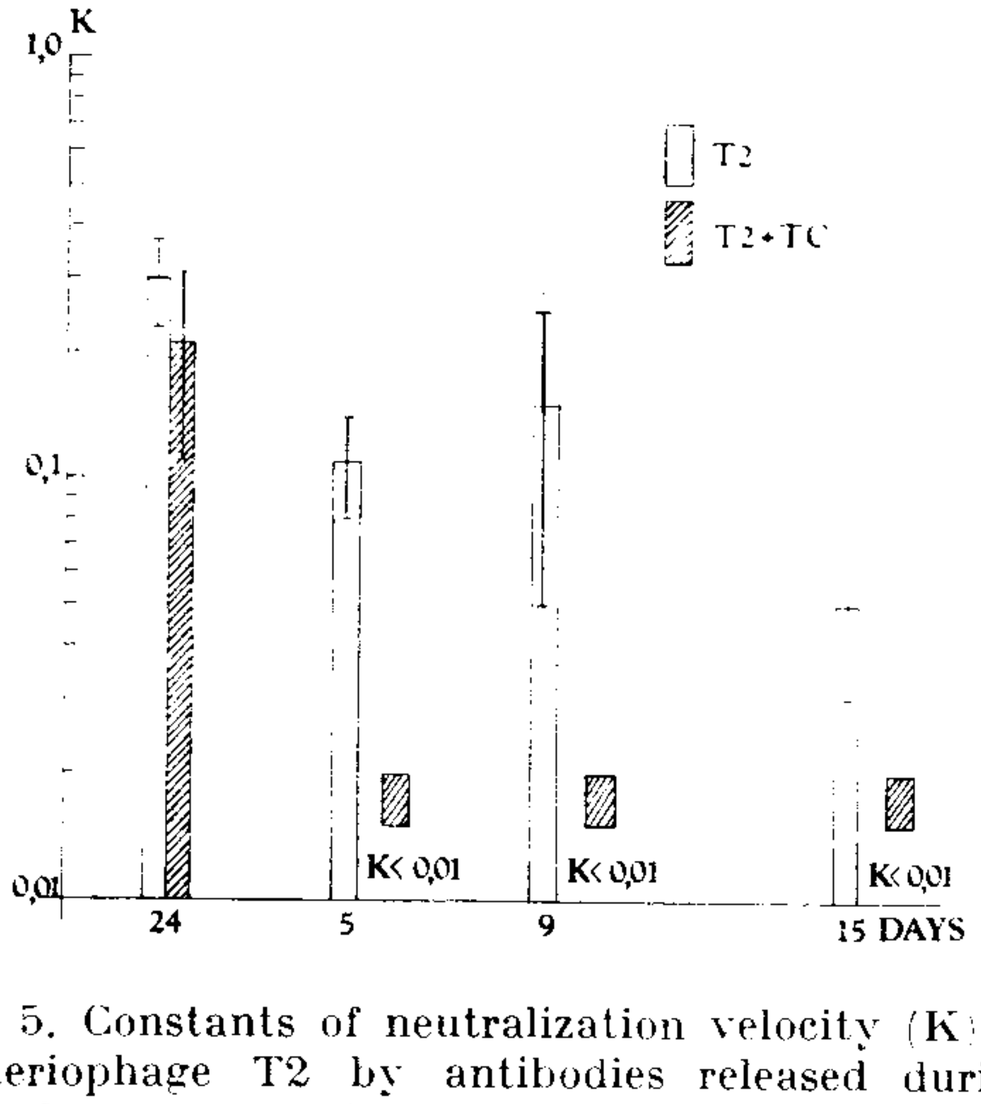

Fig. 5. Constants of neutralization velocity (K) of bacteriophage T2 by antibodies released during secondary response into media of bone marrow cell cultures. Secondary stimulus (10^8 PFU T2 per 1 ml medium) and tetracycline (1 mg per 1 ml medium) were applied *in vitro*. Open columnns: K in media without tetracycline. Closed columnns: K in media with tetracycline added at every change of medium.

logous suppression was observed in media with TC taken on day 9 and 15. In cultures of fragments of regional lymph nodes the "K" of media taken from tubes on day 5, 9 and 15 after secondary stimulation was 0.11, 0.23 and 0.09 respectively. In parallel TC-treated cultures the respective "K" values were 0.06, 0.05 and 0.00, demonstrating a significant inhibition of antibody production.

The present experiments clearly show that tetracycline (TC) even in nontoxic doses is capable of temporarily suppressing antibody production by a direct effect on antibody forming mammalian cells (especially from lymph nodes) as was first demonstrated by us[7,8] and later by *Nikolov et al.*[9]. A very interesting finding is the fact that during the secondary immune response the production of IgG is suppressed the most and that of IgM substantially less. The suppression by TC can be demonstrated *in vivo* and more clearly so in explanted tissue *in vitro*. These data are in agreement not only with the suppression of proteosynthesis in bacteria (demonstrated by the limited production of protein exotoxins in Clostridium botulinum[10,11] and streptolysin S[12]), but also repeatedly with the inhibition of proteosynthesis in mammals[13]. This probably occurs in transcription by the inhibition of the aminoacyl sRNA bond on mRNA of the ribosome complex. The inhibition of proteosynthesis in mammalian cells (and consequently in those producing antibodies) seems to be transitory and requires a substantially larger amount of TC than is necessary in attaining the same effect in the bacterial cell.

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