Specification of the immune response: its suppression induced by chloramphenicol in vitro

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(Received 23 November 1982)

Specific in vitro PFC responses to trinitophenyl conjugated to sheep red blood cells are inhibited by chloramphenicol (CAP), thiamphenicol (TAP), and diuron (DIU) by B-cell impairment. However, both mitogenic and polyclonal response to lipopolysaccharide is not affected by CAP and TAP but severely inhibited by DIU. Similarly, contact of cultured cells for the first 24 h with CAP did not much affect the 4th-day in vitro PFC response but the same incubation with DIU reduced it by 70%. Moreover, DIU used at the same concentration provoked an important mortality of cultured cells. These differences suggest that the target mechanism of CAP and TAP differs from that of DIU.

Some of the processes underlying the generation of immune specificity are now well understood. Analysis of DNA sequences of a number of light- and heavy-chain genes has suggested that a large fraction of the immune repertoire is generated, as formerly postulated by Dreyer and Bennett, by combinatorial somatic recombination between a pre-existing set of genes (1,2). The known arrangement of coding DNA sequences in the immunoglobulin genes showed that complex splicing processes are required in order to generate functional light- and heavy-chain mRNA molecules. The molecular events which underlie splicing are not understood. It has been proposed that translation of intron-encoded polypeptides might be necessary for splicing, especially during the maturation period required for specification of the immune response (3). Translation of intervening sequences located in nuclear pre-mRNA would require the existence of a nuclear protein-synthesizing machinery. Although the existence of such machinery has been suggested it remains a controversial subject (4). Its main identification was based on findings suggesting that, associated with purified nuclei, there exists a polypeptide-synthesizing machinery which is extremely sensitive to chloramphenicol, and perhaps stimulated by aurintricarboxylic acid (5). Indeed, such features would clearly separate such machinery from the cytoplasmic one which is not sensitive to chloramphenicol. It would not, however, separate from the mitochondrial protein-synthesizing machinery. This prompted us to

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reconsider the known effects of chloramphenicol on the immune response (6,7), which have been usually ascribed to inhibition of mitochondrial protein synthesis (8). In order to gain better insight into some of the processes involved in the response, we investigated the effect of chloramphenicol (and its non-toxic analogue thiamphenicol) on the specification of the immune response elicited in vitro. In addition we have compared chloramphenicol effects with the effects induced by diuron, which blocks respiration without directly inhibiting protein synthesis.

Although indirect, our results support the contention that a protein-synthesizing machinery, distinct both from cytoplasmic and mitochondrial ones, is required in order to allow specification of the immune response. This result is reminiscent of the results of Bertolini and Poggioli, who have recently found that a new compartment involving a new protein-synthesizing machinery is required for synaptogenesis (9).

Materials and Methods

Animals. Male adult DBA/2 and pathogen-free nu/nu mice (Iffa Credo, Lyon, France) were used.

Antigens and mitogen. Trinitrophenyl was conjugated to sheep red blood cells (TNP-SRBC) according to the method of Rittenberg and Pratt (10), and to polyacrylamide beads (TNP-PAA, a type-2 T-independent, or TI2, antigen) according to the method of Inman et al. (11). TNP-conjugated bacterial lipopolysaccharide (026 B6, Difco), TNP-LPS (12), was used as T-independent type-1 (TI1) antigen.

Native LPS was also used as a mitogen or polyclonal activator.

Antibiotics. The antibiotics chloramphenicol (CAP), thiamphenicol (TAP), and diuron (DIU) (Sigma) were used for the present in vitro study at concentrations of 5 or 50 μg/ml.

Spleen-cell cultures. Spleen cells from DBA/2 or nu/nu mice were washed in medium 199 and resuspended in the final RPMI 1640 medium supplemented with 10% foetal-calf serum, 2.5% pyruvate, and 5 x 10⁻⁵ M mercaptoethanol. The cell suspension (200 μl at 5 x 10⁶ cells/ml) was distributed in wells of microtiter plates together with antigen. Antigen concentration was 3 x 10⁵ cells for TNP-SRBC, or 1 μg/ml of TNP-LPS, or 1/400 dilution of TNP-PAA pellet.

Haemolytic plaque assay. At days 4 or 5, cultured spleen cells were assayed against native SRBC or TNP-SRBC + C' in liquid medium. Specific anti-TNP plaque-forming cells (PFC) were calculated by subtracting the number of SRBC plaques from the TNP-SRBC plaque number. The results are expressed as number of PFC per culture. Polyclonal response was induced by native LPS (50 μg/ml) and measured by the number of anti-TNP and SRBC PFC per culture on day 4.

[³H]thymidine (³H-T) incorporation assays. Spleen cells suspended in RPMI 1640 medium supplemented with 10% foetal-calf serum (Gibco) were cultured in triplicate microtiter plates (Falcon) (3 x 10⁶ cells/ml) for 2 days in air + CO₂ gas mixture, in the presence of LPS (50 μg/ml) or alone. Six hours before the assay, 1.5 μl of ³H-T (1 Ci/mmol) was added. The cells were harvested on a Whatman GF-83 filter, washed extensively in saline, and dried at 37°C,