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6. The discovery of diphtheria toxoid and the primary and secondary immune response

Glenny AT, Südmersen HJ. J Hyg 1921; **20**: 176–220

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Background

The routine title of the long 'note' by Glenny and Südmersen reproduced here [1] hides two of the most significant findings in theoretical and applied immunology: a brief description of diphtheria toxoid and a comprehensive account of the primary and secondary immune response. The introduction of antitoxin treatment of diphtheria was heralded, in 1896, without exaggeration as 'the most important advance of the [19th] Century in the medical treatment of acute infective disease' [2]. However, there were problems with the standardization and potency of antitoxin. The former was solved by Ehrlich who, in his analysis of toxin–antitoxin interaction, postulated the existence of 'toxoid', a non-toxic component of toxin which combined with antitoxin [3]. The latter was solved by the general observation that repeated injections of gradually increased doses of toxin induced increasingly potent antitoxins, an important basis upon which the present paper was founded.

Attempts to prevent diphtheria in humans by using small doses of toxin as a vaccine proved too dangerous [4]. Instead, toxin–antitoxin mixtures were used. However, the proportions of toxin and antitoxin had to be very carefully balanced and even so, on occasions the complex would dissociate and prove too toxic [5]. A major development, by Schick in 1913, was that susceptibility to diphtheria could be determined by inoculating small doses of toxin; in immune individuals the toxic activity was neutralized [3, 4].

Glenny and Südmersen's paper

Glenny, who joined the Wellcome Laboratories straight from school and retired as head of immunology there, had published four papers in the journal with Südmersen when this one appeared in 1921 [6]. The paper is long but well laid out. The contents are listed in 38 numbered statements (here designated s1, etc.), including interim summaries [s21, s32, s37]. Many results, not all relevant here, are recorded in tables and 'curves'; for convenience the latter are discussed here when possible.

The preliminary description of toxoid is very brief [s3, Table III], and the method used to prepare it is not described (see Comment below). Formalin treatment increased the minimum lethal dose (mld) of toxin for guinea-pigs by 10^3. However, when injected into guinea-pigs, the 'toxoid' produced antitoxin sufficient to protect against 2 mld.

The experiments on the response to 'primary' and 'secondary' stimuli are comprehensive. Guinea-pigs and rabbits were mainly used, but confirmatory experiments were done on horses, sheep, goats, cows and one immune human – Glenny [s36, Table XL]. Animals were injected with toxin–antitoxin mixtures. Any passive immunity conferred would disappear rapidly, any persistent activity was due to newly produced antitoxin.

Primary stimuli generally produced low levels of antitoxin after a latent period of $\sim$4 weeks and reached maximum titres by $\sim$10 weeks [s21, curves 1–8]. Antitoxin declined but could be detected at very low levels for up to 2 years [s10, Tables XII, XIII].

When a secondary stimulus was given, antitoxin titres increased after $\sim$4 days, with high maximum titres detectable by $\sim$10 days [s32, Table X]. Results with different animal species were essentially the same. In preliminary experiments in guinea-pigs, it was also shown that animals with very low levels of residual antitoxin produced very high levels when the second stimulus was injected 9 to $>$12 months later [s27].

What was to become the classic graphical representation of the primary and secondary response can be seen in curves 11 (guinea-pig), 14 (rabbit), 15 (goat) and 16 (horse). Perhaps the most important statement is made at the end of the introduction; 'The primary and secondary stimulus phenomena may yet be found of universal application to immunity and not limited only to antitoxin production.'

Comment

In some respects, and particularly with hindsight, the paper disappoints. Little attention is given to earlier work; only three papers are cited. It presents results and obvious conclusions but, apart from the above quotation, does not look forward. Perhaps the most surprising feature is the failure to appreciate the potential significance of the properties of toxoid. Glenny discovered toxoid in 1904 [7], and by accident [4, 6]. Apparently toxin was stored in earthenware jars too large to be autoclaved. So, they were disinfected with formalin before re-use. Residual formalin then inactivated the toxicity, but not the antigenicity, of the next batch of toxin. Why there was such delay in reporting these important observations is not known. By the time Glenny did appreciate the significance [7] the initiative had passed to Ramon, who pursued the use of toxoid ('anatoxine') for prophylaxis vigorously [3, 4, 8].

The idea that infection or vaccination left the body with some sort of memory was generally accepted. However, the different antibody classes had not then been described, and Glenny thought of the secondary response simply as quicker and more efficient than the primary; there is no suggestion that the secondary response involved an enhanced memory effect.

Historical perspective

Diphtheria

Although used extensively in France and Canada, the first toxoids produced reactions unacceptable in Britain [3, 4]. Glenny continued to work on diphtheria prophylaxis, and from 1926 developed the successful alum-precipitated toxoid [9], which was used for many years. Diphtheria prophylaxis, now using toxoids of great purity incorporated into diphtheria-tetanus-pertussis vaccine, still remains one of the major successes of preventive medicine.

The immune response

The existence of the primary and secondary immune response was soon confirmed [10] and discussed in important textbooks of immunology [11, 12]. Glenny reviewed the practical applications of immunological principles in the journal in 1945 [13]. The terms primary and secondary are still generally used, although the latter is sometimes referred to as an 'anamnestic' response. The responses are now known to be associated with different antibody classes, and this knowledge lies at the centre of much of our study of infection, in particular the design of vaccination schedules and serosurveys, and in serodiagnosis. In a wider context, Glenny and Südmersen's observations eventually led to our understanding of the complex interplay of B and T cells in the functioning of the immune system.

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