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IMMUNOLOGIC EFFECTS OF TEICHOIC ACID, AN INANIMATE BACTERIAL PRODUCT IN THE ENVIRONMENT: A REVIEW

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ABSTRACT. A variety of stable bacterial products such as lipopolysaccharide and teichoic acid are present ubiquitously in the environment. These substances have the potential for inducing extensive biological effects in animals, some of which are of great impact on the immune response. For example, injected or orally administered glycerol teichoic acid induces antibodies of unrelated specificities and may either enhance or suppress other immune responses nonspecifically. More importantly, environmental teichoic acid contained in the food of rats suppresses antibody responses to foreign erythrocytes. In view of this demonstrated suppression, the possibility exists for a dual immunologic regulatory role by bacterial substances in the environment.

LITERATURE REVIEW

In addition to the secretion of various agents which produce pathogenic effects during infection, microorganisms release a number of substances into the environment and contribute substantial residues upon dissolution. For example, gram-negative bacteria release endotoxin, a lipopolysaccharide (LPS), upon lysis after both parasitic and saprophytic growth (Nowotny 1969), and gram-positive bacteria, whether parasitic or not, secrete teichoic acids (Wicken and Knox 1975) and dextrans. These substances are stable and capable of persisting in the environment and in the body for long periods, during which time they have potential for causing extensive biological effects in animals. These effects may include hypersensitivity (Elin and Wolff 1976, Bolton and Chorpenning 1974, Frederick, Holmes and Chorpenning 1972, Martin et al. 1966), pathologic changes (Elin and Wolff 1976, Chorpenning and Dodd 1965, Ne'eman and Ginsberg 1972, Kohashi et al. 1977), lymphocyte mitogenicity (Andersson and Blomgren 1971, Elin and Wolff 1976, Beachey et al. 1979), polyclonal B cell activation (Andersson et al. 1972, Oldfather and Chorpenning 1980, Coutinho et al. 1974), specific antibody formation (Landy and Baker 1966, Decker et al. 1972, Chorpenning et al. 1979a), and alteration of unrelated immune responses (immunomodulation) (Franzl and McMaster 1968, Miller and Jackson 1973, Chorpenning et al. 1979b, Battisto and Pappas 1973). All of the bacterial products mentioned above produce immunomodulation and polyclonal B cell activation (PBA) (stimulation of different lymphocyte clones to release a variety of antibody specificities), as do a number of other polymers (table 1). In this review, the immunologic effects of glycerol teichoic acid (GTA) will be examined, as a model system, with appropriate comparisons to other bacterial products.

Teichoic acids are immunogenic constituents of gram-positive bacterial cells which consist of phosphodiester-linked residues of either ribitol or glycerol on which alanine or sugar residues may be substituted (Knox and Wicken 1973). The poly-glycerophosphate variety has been shown to frequently contain fatty acids (Wicken and Knox 1975). Recently, it has been shown that the purified, stripped backbone (fig. 1) of a bacillary GTA induces
### Table 1

<table>
<thead>
<tr>
<th>Substance</th>
<th>Source Organisms*</th>
<th>Immunomodulation**</th>
<th>Ref†</th>
<th>PBA</th>
<th>Ref‡</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>T-independent Antigens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lipopolysaccharide</td>
<td><em>Salmonella typhosa</em></td>
<td>+</td>
<td>3</td>
<td>+</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td><em>Escherichia coli</em></td>
<td>+</td>
<td>11</td>
<td>+</td>
<td>7</td>
</tr>
<tr>
<td>Purified protein derivative</td>
<td><em>Mycobacterium tuberculosis</em></td>
<td>+</td>
<td>19</td>
<td>+</td>
<td>20, 23</td>
</tr>
<tr>
<td>Glycerol teichoic acid</td>
<td><em>Streptococcus pyogenes</em></td>
<td>+</td>
<td>5, 18</td>
<td>+</td>
<td>21</td>
</tr>
<tr>
<td>Dextran</td>
<td><em>Bacillus sp.</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Levan</td>
<td><em>Lactococcus mesenteroides</em></td>
<td>+</td>
<td>2</td>
<td>+</td>
<td>6</td>
</tr>
<tr>
<td>Peptidoglycan</td>
<td><em>Corynebacterium levaniformis</em></td>
<td>?</td>
<td></td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Polysaccharide SIII</td>
<td><em>S. pneumoniae, Type III</em></td>
<td>+</td>
<td>16</td>
<td>+</td>
<td>8</td>
</tr>
<tr>
<td><strong>T-dependent Antigens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pyrogenic exotoxin</td>
<td><em>S. pyogenes</em></td>
<td>+</td>
<td>12, 13</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Enterotoxin</td>
<td><em>Vibrio cholerae</em></td>
<td>+</td>
<td>4, 15</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Enterotoxin</td>
<td><em>S. aureus</em></td>
<td>+</td>
<td>17, 22</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*Other organisms may also produce the substance in question.

**Either suppression or enhancement of unrelated immune responses may occur, depending on dosage and route.

†References:
1. Andersson et al. 1972
2. Bartraro and Pappas 1973
3. Bradley and Watson 1964
4. Chisari et al. 1974
5. Chorpenning et al. 1979
6. Coutinho et al. 1974
7. Coutinho and Möller 1973a
8. Coutinho and Möller 1973b
9. Dziarski 1978
10. Dziarski and Dziarski 1979
11. Franzl and McMaster 1968
12. Hanna and Watson 1968
13. Hanna and Watson 1973
14. Holton and Schwab 1966
15. Kately et al. 1975
16. Klaus et al. 1975
17. Langford et al. 1978
18. Miller and Jackson 1973
19. Möller and Kashiwagi 1972
21. Oldfather and Chorpenning 1980
22. Smith and Johnson 1975
23. Sultzer and Nilsson 1972

both humoral and cellular immunity (Chorpenning et al. 1979a) and is capable of altering unrelated immune responses (Chorpenning et al. 1979b). It is particularly interesting that many gram-positive bacteria are prevalent in the normal flora of mammals and in the environment, while 'natural' humoral and cellular responses to GTA occur regularly in many animals (Decker et al. 1972, Frederick et al. 1972, Bolton and Chorpenning 1974) without the presence of an overt antigenic stimulus. It has been demonstrated in rats that the actual stimulus is environmental GTA present in the diet (Rozmiarek et al. 1977). Newer evidence (below) indicates that this environmental teichoic acid suppresses immune responses to unrelated antigens.

LPS is also prevalent in the environment and is immunogenic. While most antigens usually require the presence of T lymphocytes as well as the antibody-synthesizing B lymphocyte and the macrophage for an antibody response, LPS was shown to be capable of inducing specific antibodies in the absence of T cells (Andersson and Blomgren 1971, Möller and Michael 1971). Interestingly, large doses of LPS were shown also to induce non-specific stimulation of B cells, polyclonally, producing antibodies of a variety of specificities (Andersson et al. 1972). This T-independence was later demonstrated by
dextran and polyclonal B cell activation was produced by dextran sulfate (Coutinho et al. 1974), as well as by certain other polymeric bacterial products (table 1). In view of these effects, the GTA polymer was examined for T-dependence, using T lymphocyte depleted Fisher 344 rats (Oldfather and Chorpenning 1980). Adult rats were thymectomized, lethally X-irradiated (600R), and reconstituted with bone marrow from inbred (syngeneic) rats of the same strain. These rats (ATxXBM) were shown to be depleted of T cells by their lack of splenocyte responses to T cell mitogens (Concanavalin A and phytohemagglutinin) and to the T-dependent sheep red blood cell (SRBC) antigen. Yet, antibody responses to soluble purified GTA measured in both spleen and serum were equal to those of normal and sham-operated rats, indicating that the antigen is T-independent. GTA-injected animals also exhibited antibody responses of other specificities when spleen cells were examined by the hemolytic plaque test (Oldfather and Chorpenning 1980). Significant antibody-secreting cells were demonstrated for erythrocytes of several species, as well as for the dinitrophenyl determinant. This nonspecific (PBA) stimulation by GTA (table 2), although not unexpected in view of its induction by LPS and other polymers, is of considerable significance in view of the stability and ubiquitous presence of GTA. It raises questions regarding a possible relationship between immunomodulation and nonspecific responses to PBA's, and regarding their significance in protection against infection or in induction of inflammatory diseases (Claggett and Engel 1978).

**Table 2**

Polyclonal B lymphocyte responses to glycerol teichoic acid

<table>
<thead>
<tr>
<th>Specificity**</th>
<th>PFC/10^7 Splenocytes*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GTA</td>
<td>141 ± 20</td>
</tr>
<tr>
<td>SRBC</td>
<td>96 ± 13</td>
</tr>
<tr>
<td>DNP</td>
<td>57 ± 17</td>
</tr>
<tr>
<td>CRBC</td>
<td>51 ± 13</td>
</tr>
<tr>
<td>HRBC</td>
<td>45 ± 14</td>
</tr>
<tr>
<td>PBS</td>
<td>8 ± 3</td>
</tr>
</tbody>
</table>

*Single i.v. injection of 400 µg given 4 days before testing.
**Specificity of erythrocytes used in hemolytic plaque assay (PFC). All were native antigens except for dinitrofluorobenzene-treated SRBC (DNP) and GTA-coated SRBC.

The relationship between specific and nonspecific responses to PBA’s has not been sufficiently investigated. These responses were studied in cultures of Balb/c mouse splenocytes to which GTA alone was added (Young and Chorpenning 1980). The kinetics were different for hemolytic plaque-forming cells (PFC) of SRBC and GTA specificities. At an equal dose (0.3 ug GTA per 10^6 cells), a peak SRBC response occurred at 2 days while the peak GTA response was seen at 4 days (usual for specific PFC responses in this species). Although both types of response were T-independent, the presence of nonspecifically stimulated lymphocytes augmented the specific anti-GTA response. These results suggest that separate mechanisms exist for the specific and nonspecific responses elicited by GTA. Perhaps an even more convincing observation suggesting that the 2 kinds of response are separate was that made in the Fisher rats (Oldfather and Chorpenning 1980). No PBA effect was seen in this strain when the animals were normal, yet good specific GTA responses were obtained. PBA responses were observed, however, in ATxXBM rats, indicating that T cell depletion removed some regulatory control of the PBA effect. Since there was no such control in the specific
response, it would appear that the two are distinct.

The effect of GTA on unrelated responses was originally observed as suppression of responses to sheep red blood cells (SRBC) in the C3H/He mouse (Miller and Jackson 1973, Miller et al. 1976). This effect was confirmed in C3H/HeJ mice and it was shown that it could be produced with lipid-free GTA (Chorpenning et al. 1979b). It was also shown that modulation of the response to SRBC could be demonstrated in vitro with C3H/HeJ or BDF1 mouse splenocytes and that the direction of modulation was dose-dependent (Lynch and Chorpenning 1978). Thus, low doses induced enhancement of the response to SRBC and high doses induced suppression, effects also reported for LPS (Hoffman et al. 1975). Further studies in C3H/HeJ splenocyte cultures using cell populations depleted of adherent cells demonstrated that enhancement by GTA is induced through stimulation of the nonadherent cell population while suppression (at high doses) is induced through the adherent splenocytes (Lynch and Chorpenning 1979). When peritoneal macrophages (adherent cells) were substituted for adherent splenocytes, suppression did not occur and dose-related enhancement by GTA was equal to that seen in adherent cell depleted cultures. Therefore, 2 facts seem clear at this point. In immunomodulation, GTA acts directly or indirectly on at least 2 cell populations, one suppressing the response and the other enhancing it. Whether the latter effect is entirely due to GTA being a PBA or involves some additional mechanism is not yet clear. It is possible that suppression results from activation of a suppressor cell population(s) by GTA, as first suggested for other antigens by Gershon and Kondo (1971).

LPS also modulates unrelated immune responses in a manner related to dosage and route, producing either suppression or enhancement depending upon these variables (Franzl and McMaster 1968). Thus, substances of bacterial origin appear to play a dual regulatory role in the immune response, acting as either activators or suppressors.

Probably, the most significant observation regarding GTA was the demonstration that environmentally acquired GTA (in food) suppresses immune responses in Sprague-Dawley rats (Oldfather and Chorpenning 1981). When rats were raised from weaning on a GTA-free diet they yielded over 4-fold higher responses to injected SRBC than did rats fed a conventional diet (table 3). Responses to chicken red blood cells were also suppressed in conventionally fed animals as compared to rats deprived of GTA. Suppression could be reinstated in the GTA-deprived rats by feeding soluble purified GTA and the degree of suppression was dose-related. In view of the dose-response

<p>| Table 3: Effect of dietary GTA on responses to sheep erythrocytes* |
|---------------------------------|----------------|----------------|----------------|</p>
<table>
<thead>
<tr>
<th>Diet</th>
<th>GTA Source</th>
<th>Hemolysin (GTA)**</th>
<th>GTA-PFC*</th>
<th>SRBC-PFC**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regular</td>
<td>Environment</td>
<td>26 ± 2.8</td>
<td>1.3 ± 0.8</td>
<td>351 ± 47</td>
</tr>
<tr>
<td>GTA-free</td>
<td>None</td>
<td>0</td>
<td>0</td>
<td>1624 ± 281</td>
</tr>
<tr>
<td>GTA-free</td>
<td>200 ug/wk orally</td>
<td>1259 ± 330</td>
<td>2.6 ± 0.4</td>
<td>402 ± 125</td>
</tr>
</tbody>
</table>

*Data abstracted from Oldfather and Chorpenning, 1981.

**Mean 50% hemolysis titer of antibodies specific for GTA (± S. E. M.), 5-7 animals per group.

*Mean numbers of PFC (antibody-secreting cells) of GTA specificity (± S. E. M.) per 10⁶ splenocytes in groups of 5-7 animals.

**Mean numbers of PFC of SRBC specificity (± S. E. M.) per 10⁶ splenocytes in groups of 5-7 animals, each injected i.p. with 1 ml of a 10% suspension of SRBC.
relationship and the earlier clear cut evidence that appropriate amounts of injected GTA suppress immune responses, it appears that environmental GTA acts suppressively. Bolton (1980) also observed a significant difference between SRBC responses of GTA-deprived rats and those on the usual diet, but that was not the major objective of his investigation and the comparability of the 2 groups of animals was not clarified. Also, he did not report as great a difference as that seen in the foregoing investigation. In addition, he observed that injection of GTA-anti-GTA complexes was required to induce suppression, which is at variance with the observations of Oldfather and Chorpenning (1981), where dose-related suppression was produced when GTA-deprived, antibody-free rats were fed purified GTA. Very likely, the difference in results were due to differences in dosage (200 μg/wk vs. 40 μg total dose) and/or route and timing.

DISCUSSION AND CONCLUSIONS

A variety of stable bacterial products, which are released into the environment, are capable of producing significant biological effects. Prominent among these effects are T-cell independent immunogenicity, polyclonal B cell activation, and immunomodulation. Several of these substances, such as LPS and GTA exhibit polyclonal activation when large doses are injected into animals, resulting in elicitation of a variety of antibodies which are reactive with a wide range of antigens. It has been suggested that such antibodies are protective, constituting a primitive form of immunity (Petit and Unanue 1974). On the other hand, large doses of LPS and GTA suppress specific responses to unrelated T-dependent cellular antigens, such as sheep red cells. It would appear that constant environmental exposure to these ubiquitous substances would lead to build-up of high doses and that both PBA and suppression of specific immune responses to T-dependent bacterial antigens would ensue. Based on the experiments with GTA described above, this does appear to be the case, although in these experiments erythrocyte surrogates were employed rather than bacterial cells. Furthermore, a low-level background of numerous antibody specificities is regularly observed in many species, which seems somewhat large and diverse to be accounted for by antigenic crossreactions. Stimulation by PBA's in the environment could account for these. Since it appears that the nonspecific (PBA) and specific responses involve different B cell populations (work cited above and Coutinho et al. 1976), the simultaneous PBA and suppressive effects on a single antibody specificity are not inconsistent. Furthermore, according to Gronowicz and Coutinho (1974) and Möller et al. (1976), the B lymphocytes undergoing PBA are pre-existing subpopulations for each PBA agent which need not proliferate appreciably during the event, while the B-cell clone responding to a specific antigenic stimulus is a different one and does proliferate. The cells involved in GTA suppression appear to be a still different population from those above. Therefore, proliferation under a continuing stimulus of specific antigen could override the suppressive effect. In such a circumstance, PBA effects from environmental stimulation could furnish early protection while the later specific response would subsequently confer a higher level of immunity.

LITERATURE CITED


Beachey, E., H. James, B. Dale, S. Grebe, A. Ahmed, W. A. Simpson and I. Ofek 1979


Ne’eman, N. and I. Ginsburg 1972 Cell sensitizing antigen of group A streptococci. II. Immunological and immunopathological properties.


Oldfather, J. W. and F. W. Chorpenning 1980 Role of T lymphocytes in antibody responses to glycerol teichoic acid. Ohio J. Sci. 80: 45 (Progr Abst.)


