Immunotoxicology of Foodborne Substances: An Overview

D. L. ARCHER

Department of Health, Education, and Welfare
Public Health Service, Food and Drug Administration
Division of Microbiology, Cincinnati, Ohio 45226

(Received for publication June 8, 1978)

ABSTRACT

The current status of research concerning immunotoxicology of foodborne substances is discussed. Several food additives and bacterial toxins interact directly with cells involved in the immune response and could interfere with natural immunoregulation in the gut. Specialized antibodies produced locally in the gastrointestinal tract help facilitate enzymatic degradation of the substances with which they react by retaining them along the mucous lining; this process is known as immune exclusion. Interference with local production of intestinal antibodies could lead to loss of local immunity and immune exclusion of large, potentially immunogenic proteins. Consequences of chemically induced loss of immune exclusion could include increase in incidence of autoimmune diseases, gastrointestinal allergies, toxigenic diarrheas, and pathogenic invasion through the gut wall. The need for more research is apparent.

The thrust of current research in food toxicology has, for the most part, been directed at methods for detecting chemical mutagens and/or carcinogens. In vitro methods, various cell culture methods, and tests done with Drosophila and rats have been used to screen for chemicals that are potentially mutagenic and possibly carcinogenic for man (8). This area of research is extremely important and has yielded much useful information. Many mutagenic or carcinogenic changes (transformations) in mammals are dealt with effectively by the body’s own natural defense mechanism, the immune system. This extremely complex system is responsible for the well-being of the human animal in many ways, including (a) rejection and destruction of both living and nonliving foreign material, (b) neutralization of pathogens such as viruses, mycoplasma, bacteria and their toxins, and other parasites, and (c) recognition and destruction of cells that have undergone transformation to a malignant state. In a healthy individual, the immune system carries out its protective functions with remarkable efficiency. When the individual’s immune system is compromised by congenital defects, infection, or nutritional factors, a multitude of serious disorders can result (reviewed in 1,2,3). The purpose of this article is to provide an overview of past and current work concerning the possible immunologic consequences of ingesting foodborne immunosuppressive chemicals or chemicals capable of altering immune regulation. Food additives, some natural constituents, and bacterial toxins will be discussed; pesticide residues have been reviewed elsewhere (30).

THE IMMUNE SYSTEM

Figure 1 illustrates some of the cells and interactions involved in the various types of immune responses. The cells from which immunocompetent cells are derived are termed “stem cells.” These migrate from the bone marrow and, under the influence of various organs, become lymphocytes in one or the other of the two major compartments of the immune system. Stem cells influenced by the thymus become the T-lymphocytes. These can be further divided into subpopulations, some of which act as (a) killer cells that are specifically cytotoxic to other cells (foreign or transformed) to which they are sensitized, (b) helper cells that are required for an antibody response to many antigens, and (c) suppressor cells that can inhibit formation of antibody, a normal negative control mechanism, via soluble mediator(s). The other major compartment is made up of the B-lymphocytes. These arise from stem cells that come under the influence of the so-called bursa equivalent (from studies in chickens). In man, the bursa equivalent is believed to be fetal liver and spleen, and bone marrow. B-cells have surface antibodies specific for antigen and are the precursors to antibody-forming cells (AFC). When triggered by their appropriate antigen, and possibly after interaction with the regulatory T-cells, B-cells mature into plasma cells that produce antibody directed against the stimulating antigen. Not shown in Fig. 1, but of great importance to immune mechanisms, are the monocytes and macrophages. Besides their duties as scavenger cells and processors of antigens, regulatory functions have recently been ascribed to macrophages (9,10). The interactions among the various cells are not...
yet fully understood, but they are known to involve many soluble products (lymphokines), the list of which is rapidly expanding (reviewed in 3).
The results obtained with gallic acid, a metabolite of the food additives propyl gallate and tannic acid and a natural constituent of some foods, are particularly interesting and exemplify the ability to ascertain the affected cell type using the M-D system. The ability of gallic acid to suppress the AFC response to sheep erythrocytes was shown to be reversed by gallic acid blocked functions of T-lymphocytes that were later shown to be achieved by embryo-propyl gallate and tannic acid and a metabolite of gallic acid (12).

Figure 3. Direct plaque formation by IgM-secreting plasma cells stimulated in vitro. The reaction of antigen (erythrocytes or antigen-coupled erythrocytes) with antibody (IgM) secreted by plasma cells in the presence of complement causes hemolysis. The clear zone around the plasma cell is a plaque.

Calcium cyclamate was not directly immunosuppressive in the M-D test. This result is contrary to in vivo feeding studies involving rabbits. In those tests, rabbits fed diets containing 5% calcium cyclamate for 150 days demonstrated reduced antibody responses to antigenic challenge (14). It was not demonstrated whether this reduction was due to direct effects on immunocompetent cells, an indirect effect of involvement of another organ, or hormonal imbalance.

Spiers et al. (27) have recently proposed an elaborate model system for evaluating the effect of potentially immunosuppressive compounds in mice. This system employs a vaccine consisting of DPT (diptheria, tetanus toxoid, pertussis) with S3 pneumococcal polysaccharide added; this mixture permits assessment of the effect on both T-lymphocyte-dependent and T-lymphocyte-independent antibody responses. The effect of an established immunosuppressant, cyclophosphamide, was studied in this system (27). Time of administration of either the antigen cocktail or immunosuppressant could be varied, giving the system great flexibility. This predictive tool should prove to be valuable for determining the immunosuppressive potential of various compounds.

BACTERIAL TOXINS

Bacterial toxins have also been shown to disrupt normal immunoregulatory mechanisms. Staphylococcal enterotoxins A and B, for example, can exert an immunosuppressive effect on antibody formation in vitro (25). This effect is thought to be mediated by soluble T-lymphocyte products (lymphokines), which, once produced, can spread from the toxin-affected T-cell to exert their suppressive effect on nearby cells. These studies were carried out using mouse cells in vitro. Enterotoxin A in amounts as small as $10^{-6}$ µg/ml has been recently shown to activate human peripheral lymphocytes to divide and to produce large amounts of immune interferon, a lymphokine (personal communication, M. L. Langford, G. J. Stanton, H. M. Johnson, U. of Texas Medical Branch, Galveston, Texas. 1978). Sub-emetic doses of these toxins could have a significant effect on the normal immunoregulatory processes in the gastrointestinal tract. Induction of suppressor T-lymphocytes, a normal immunoregulatory process, can be reversed by cyclic adenosine monophosphate (cyclic-AMP) or its inducers in vitro (17). Toxins such as cholera toxin and possibly those from other gram-negative organisms are potent inducers of cyclic-AMP activity (12). The effects of toxin-induced disruption of immunoregulation require in vivo confirmation. Although effects on the immune response would be more difficult to assess
Table 1. The effects\(^a\) of some common phenolic food additives or food additive metabolites on the Mischell-Dutton system.

<table>
<thead>
<tr>
<th>Compound</th>
<th>(R_1)</th>
<th>(R_2)</th>
<th>(R_3)</th>
<th>(R_4)</th>
<th>(R_5)</th>
<th>Functional groups</th>
<th>90% PFCSD</th>
<th>50% VRD</th>
</tr>
</thead>
<tbody>
<tr>
<td>(p)-Hydroxybenzoic acid</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>COOH</td>
<td>H</td>
<td>50 &gt;200</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>Methyl paraben</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>COOCH(_3)</td>
<td>H</td>
<td>25 &gt;200</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>Propyl paraben</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>COO(CH(_2)(_2))(_3)H</td>
<td>H</td>
<td>50 50</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>Vanillin</td>
<td>OH</td>
<td>OCH(_3)</td>
<td>H</td>
<td>CHO</td>
<td>H</td>
<td>stimulatory &gt;200</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>Vanillic acid</td>
<td>OH</td>
<td>OCH(_3)</td>
<td>H</td>
<td>COOH</td>
<td>H</td>
<td>stimulatory &gt;200</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>Butylated hydroxyanisole</td>
<td>OH</td>
<td>C(CH(_2)(_3))</td>
<td>H</td>
<td>OCH(_3)</td>
<td>H</td>
<td>50 &gt;200</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>2-isomer</td>
<td>OH</td>
<td>C(CH(_2)(_3))</td>
<td>H</td>
<td>OCH(_3)</td>
<td>H</td>
<td>50 &gt;200</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>Butylated hydroxyanisole</td>
<td>OH</td>
<td>C(CH(_2)(_3))</td>
<td>H</td>
<td>OCH(_3)</td>
<td>H</td>
<td>50 &gt;200</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>3-isomer</td>
<td>OH</td>
<td>H</td>
<td>C(CH(_2)(_3))</td>
<td>OCH(_3)</td>
<td>H</td>
<td>25 &gt;200</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>Butylated hydroxytoluene</td>
<td>OH</td>
<td>C(CH(_2)(_3))</td>
<td>H</td>
<td>CH(_3)</td>
<td>H</td>
<td>50 150</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>Gallic acid</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>COOH</td>
<td>H</td>
<td>1-2 &gt;200</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>Propyl gallate</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>COO(CH(_2)(_3))(_3)H</td>
<td>OH</td>
<td>5 50</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
<tr>
<td>Pyrogallol</td>
<td>OH</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>OH</td>
<td>5 50</td>
<td>90% PG/C</td>
<td>50 PG/C</td>
</tr>
</tbody>
</table>

\(^a\)Suppression of the in vitro antibody response to sheep erythrocytes is presented as the dose of test compound (µg/culture) which resulted in >90% suppression of the PFC response (90% PFCSD). Toxicity was assessed by trypan blue exclusion and is presented as the dose of test compound (µg/culture) which resulted in a 50% viability reduction (50% VRD) at the end of the 5 day culture period. Control anti-SRBC PFC responses ranged from 10,000 to 25,000 PFC/culture and were background-corrected before the mean was calculated. All compounds were added to cultures at the time of antigen addition.


than overt symptoms of intoxication (vomiting or diarrhea), the potential hazards posed are great and will be discussed later.

**PRINCIPLE OF IMMUNE EXCLUSION**

The major antibody in all secretions along the mucous membranes is a specialized antibody, secretory IgA (sIgA), which is a dimeric antibody composed of two 7S IgA molecules coupled by a joining chain (J chain) and finally coupled to a secretory component (SC). The final structure, sIgA, is released into the mucus covering the intestinal villi, the glycolalcalyx. This specialized antibody is resistant to enzymatic degradation by intestinal enzymes. In a series of experiments, Walker and his associates have demonstrated a novel role for sIgA. Intact proteins were thought to be able to traverse the intestinal barrier in newborns but not in adults. Warshaw et al. (38) showed that immunogenic quantities of bovine serum albumin could traverse the intestine of adult rats. Up to 2% of the administered protein entered the intestinal lymphatics, and subsequently, the general circulation in an unaltered form. Using an everted gut sac technique, Walker et al. (36,37) proposed that intestinal antibody complexes with undigested proteins and retains them in the mucous lining, thus increasing their chances for degradation by intestinal proteases. Walker et al. (35) had previously shown that oral immunization decreased uptake of the immunizing antigen, but not of unrelated antigens; this result suggested that local immunity interfered with protein uptake in a specific manner. Thus intestinal antibodies may play a role in exclusion of intestinal antigens from either dietary or bacterial sources (detailed in Fig. 4). In a recent review of intestinal antibodies, Walker and Isselbacher (34) pointed out that the mucous barrier of the intestine, with its secretory antibodies, has become known as "antiseptic paint" to describe its function. This function is most pronounced in premature and newborn infants whose sIgA AFC are not yet functional. As pointed out by Walker and Isselbacher (34), allergy-prone infants showed an increased incidence of skin sensitivity to a variety of allergens when exposed before sIgA appearance as compared with infants exposed after sIgA appearance. The earlier that cow's milk was introduced to infants, the higher the circulating antibody level to milk proteins (32).
The incidence of autoimmune disease in persons with selective IgA deficiency is very high (2,3). Temporary loss of immune exclusion could lead to autoimmune disorders. Antigenic materials crossing the normally exclusive intestinal barrier could induce production of autoantibodies directed, as a result of cross reactivity, to self antigens. Thus, loss of immune exclusion could contribute to an increased incidence of diseases such as systemic lupus erythematosus, rheumatoid arthritis, and thyroiditis. Increased uptake of antigenic material may also sensitize an individual to that substance so that, on second exposure to the antigen at a later time, an allergic reaction may occur (reviewed in 22). Gastrointestinal allergies present a complex clinical picture and are poorly understood; epidemiological data are therefore difficult to obtain. The normal flora of the gut consists of potentially toxicogenic bacteria and viruses; temporary increase in gut permeability due to a local immunosuppressive event could allow these organisms or their toxins to establish infection or to elicit gastrointestinal disturbances. In vivo studies with mouse models have shown that immunosuppressive drugs facilitated the invasion of Pseudomonas aeruginosa across the intestinal barrier (24), resulting in the death of the mouse. Malignancies have also been connected with selective IgA deficiency (3). There are several mechanisms by which the body screens for abnormal cells; chemicals affecting a variety of cells or cellular interactions of the immune system could disrupt this normal immunologic screening. A full discussion of all possible consequences of loss of local gut immunity cannot be presented here. The above examples represent only a partial list of the possible consequences of ingestion of an immunosuppressive compound. Few if any epidemiological studies have been done correlating food additive intake with any of the disorders presented.

Another aspect of local immunity that must not be overlooked is the interferon system. Interferons act on the host cell to prevent virus replication and are a major part of the body’s defense against viral infections (reviewed in 13,15). Interferons are produced in response to provocation of any cell by virus or viral nucleic acid (Type I or virus type interferon), or by lymphocytes in response to specific antigens or non-specific mitogens (Type II or immune interferon). The two types of interferons differ chemically, and much more is known about the virus-type interferon. Both types of interferon are immunosuppressive (16,26), and immune interferon relates quantitatively to suppressor T-cell activity (17). The role of interferons as immunoregulatory molecules has not been ascertained, but it was recently shown that a food additive metabolite could block production of immune interferon and suppressor cell activation concomitantly (6). Interferons also express their antiviral function along mucosal surfaces, and much remains to be done to determine the effects of ingested chemicals on production and function of interferons.

In summary, chemicals that can react with any
component (lymphoid cell or soluble mediator) of the immune response require closer scrutiny to determine their immunosuppressive potential. Several compounds have been shown to possess immunosuppressive activity in an in vitro mouse system (4-7). In vivo systems must be utilized to determine whether there is a correlation between in vitro results and in vivo immunologic events, particularly in the gastrointestinal tract. As pointed out in a recent review by Vos (30), the importance of the immune system has thus far been underestimated in toxicologic research; the few chemicals that have been shown to be immunosuppressive may be only the “tip of the iceberg.”

REFERENCES