Lipids and Immune Function

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Abstract

There is in vitro and in vivo evidence to suggest that dietary lipids play a role in modulating immune function. A review of the current literature on the interrelationships among dietary lipids, blood cholesterol levels, immunosuppression, and tumorigenesis makes for a very strong argument that (a) immunosuppression may be causally related to lymphoproliferative disorders, as well as to tumorigenesis and (b) diets high in polyunsaturated fat, relative to diets high in saturated fat, are more immunosuppressive and are better promoters of tumorigenesis. The effects of dietary fat on immune function seem to be mediated through its component parts, the unsaturated fatty acids, specifically linoleic, linolenic, and arachidonic. It is not clear how these components affect immune function. Several studies suggest that one effect is mediated by altering the lipid component of the cell membrane and thus its fluidity; the more fluid the membrane, the less responsive it is. Thus, fluidity of both immune cells and those to be destroyed or protected may be affected. The effects of saturated as well as unsaturated fatty acids may be mediated by modulating serum lipoprotein levels, prostaglandin metabolism, and cholesterol concentrations and metabolism.

Of the several working groups at this Workshop, one deals with the relationship between lipids and the immune system. There is in vitro and in vivo evidence to suggest that dietary lipids play some role in regulating or modulating immune function. This offers the promise that by changing dietary lipids one may be able to modulate immune function and thus alter an individual's susceptibility or resistance to diseases associated with altered immune competency; e.g., immunosuppression and lymphoproliferative diseases. It is not yet generally accepted that immunosuppression may be causally related to solid tumors as well. However, a review of the current literature on the interrelationships among dietary lipids, blood cholesterol levels, immunosuppression, and tumorigenesis makes for a very strong argument that (a) immunosuppression may also be causally related to tumorigenesis, as well as to lymphoproliferative disorders and (b) diets high in polyunsaturated fat, relative to diets high in saturated fat, are both immunosuppressive and promoters of tumorigenesis. However, since neither the nutritionist nor the immunologist is certain as to what causes cancer or what cell types in the immune system or within the nonimmune host defense system protect the individual from malignancy, this aspect will not be discussed in detail. It is our aim, however, to discuss briefly the subject of lipid effects on immune function and provide a cogent argument that altered or depressed immune function may act as a promoter in or is causally related to carcinogenesis. To begin, we must define the terms "promoter" and "initiator" as they relate to carcinogenesis. Here, the word "initiator" is used to refer to any insult (addition or withdrawal of a substance, be it chemical, viral, nutrient, etc.) which results in neoplasia, irrespective of time. Nonnutrients or chemicals have been shown to initiate cancer. On the other hand, anything that decreases the time at which tumors appear or that increases numbers of tumors or enhances metastases is considered a promoter. There is no evidence that any nutrient, essential or nonessential, fed in deficient or excess amounts has ever initiated cancer, although they have been shown to enhance or promote carcinogenesis (62). The available evidence also indicates that dietary manipulations that promote tumorigenesis are immunosuppressive. All of this would suggest that immunosuppression may well be causally related to neoplasia. For a recent review of this subject, see a publication by Posner et al. (51) and others (1, 2, 4-6, 8-11, 62).

It is our opinion that the current data on this subject indicate that diets high in unsaturated fat have been shown to be good promoters of chemical carcinogenesis and are also immunosuppressive relative to diets high in saturated fat (11, 31, 32, 37, 38). Before discussing available data on lipid effects on immune function, a review of some basic immunology, although brief, is in order. The immune system is composed of a variety of cells including T- and B-cells (cellular and humoral immunity), subsets of these cells, lymphokines, serum factors, complement, macrophages, and interactions of these various cell types. Subsets of T-cells function either as helper, suppressor, or killer cells. Whether the killer cells kill directly, through interaction with macrophages, or in concert with macrophages which can also kill or lyse cells is not always clear. B-cells, the antibody-producing cells, in some instances require the interaction of T-helper cells or in other cases are inhibited by T-suppressor cell activity, presumably by suppressing T-helper function. Other B-cells are independent of T-cell influence; this depends upon the antigen which determines T-cell intervention. In recent years, another cell type has been described which is capable of lysing hemopoietic and tumor cell lines in vitro. These cells, which are called NK cells, are neither T-cells, nor macrophages, nor B-cells. These cells have been categorized as M-cells or marrow-dependent cells and may simply represent one subset of the M-cell series. The NK cell seems to be responsible for the rejection of hemopoietic allografts, genetic resistance against certain oncogenic and nononcogenic viruses, and resistance against certain intracellular bacteria (33). For an excellent review of basic immunology, the reader is referred to a recent publication by Kumar (33) and a text by Roitt (54).

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3 The abbreviations used are: NK cells, natural killer cells; PUFA, polyunsaturated fatty acids; LDL, low-density lipoproteins; HDL, high-density proteins.
A test commonly used to assess immune function in a number of research laboratories is the determination of lymphocyte blast transformation. One can measure the response of lymphocytes to certain mitogens. The subsequent synthesis of DNA can be followed by incubating isolated lymphocytes with tritiated thymidine. Two mitogens commonly used for assessing T-cell responsiveness are concanavalin A and phytohemagglutinin, whereas lipopolysaccharide is used to assess B-cell responsiveness. The amount of tritiated thymidine taken up provides some assessment of the immune system. The T-cell responding in the in vitro system is predominantly a T-helper cell.

**Dietary Fat and Immune Responses**

Using similar techniques, a number of laboratories have demonstrated that diets relatively high in polyunsaturated fat inhibit T- but not B-cell responsiveness to mitogen stimulation (11, 37, 41–43, 45). Optimal blast transformation requires the presence and cooperation of macrophages for processing and presentation of antigen to the lymphocyte. Therefore, decrease in lymphocyte blast transformation as a result of some nutritional alteration or insult may not be due to T- or B-cell defects but to aberrations within the macrophage.

Several studies have shown that an increased concentration of cholesterol within the macrophage inhibits its phagocytic process (7, 24, 25) and therefore its ability to process and kill effectively or optimally.

Other studies have shown that diets high in polyunsaturated fat or the administration of PUFA result in the loss of an animal’s ability to reject skin allografts (41, 52, 60). Also, PUFA have been used as adjuncts to immunosuppression in renal transplant patients (37, 39, 40, 61) who are at high risk for neoplasia. Whether these effects of PUFA are mediated through metabolic or structural aberrations in T-, B-, or M-cells, in macrophages, or in other cell types (production of complement) is not clear. Thus, the changes in immune function that occur with changes in dietary fat (unsaturated versus saturated) may be mediated by changes in serum or cell cholesterol levels. Changes or reductions rather than absolute levels of serum or cell cholesterol may play the major role in modulating not only immune function but the rate, induction, or latency period of chemically induced carcinogenesis. In almost every animal study, diets high in PUFA are better promoters of tumors than are diets high in saturated fat (11, 15–17). Diet regimens used to lower serum cholesterol level, such as diets high in unsaturated fat, appear to place the host at greater risk of cancer, an observation supported by some recent findings in humans (22, 50).

In addition to other regulating mechanisms, there is evidence that fatty acids also influence cholesterol synthesis and catabolism and thereby affect total body cholesterol pools. Further, there is general agreement that the lowering of serum cholesterol associated with polyunsaturated fat is attributed to the essential unsaturated fatty acids linoleic, linolenic, and arachidonic acids, the same acids which when fed or ingested have been shown to be immunosuppressive (30, 38, 41, 45, 49), to result in defects in the rejection of skin allografts (41, 44, 52, 60), and to be useful as adjuncts in the immunosuppression regimen of renal transplanted patients (37, 39, 40, 61).

The point to be made is that the quantity of unsaturated fatty acids fed and not serum cholesterol levels per se, although they will be affected by the quality of fat, appear to correlate best with most of the observed effects on immune function, in the promotion of carcinogenesis in animal feeding experiments, and in human clinical trials (17, 50, 51). For example, Kos et al. (32) found no effect of hypercholesterolemia on the response of T- or B-cells to mitogen, no effect on the tumoricidal activity of peritoneal macrophages, and no effect on the survival of mice inoculated with cells of a syngeneic methylcholanthrene-induced fibrosarcoma or the Lewis lung carcinoma. In addition, Kos et al. (32) calculated the ratio of the number of regressions of tumor to the number of mice challenged and found 0/10 and 1/10 for the normocholesterolemic and hypercholesterolemic mice, respectively. The study by Kos et al. (32) was poor in terms of nutritional design since one group of control mice was fed a commercial chow, which not only varies in composition from batch to batch but was, unfortunately, different in the content of fat, cholesterol, cholic acid, protein, vitamins, etc., from the quasipurified diet fed the experimental mice that had been made hypercholesterolemic. Nonetheless, Kos et al. (32) did demonstrate that hypercholesterolemic mice were more susceptible to infectious disease which was associated with a decreased antibody response to sheep erythrocytes in vivo. As in the case of the T-cell, the B-cell response to mitogen was not affected by hypercholesterolemia, suggesting that macrophages may have been affected rather than B-cells per se. This observation is supported by several other studies suggesting that hyperlipidemia adversely affects macrophage function (7, 24, 32). In any case, 2 observations should be noted in the study of Kos et al. and from another similar in design (34, 35): (a) the fat fed was lard, a relatively highly saturated animal fat which was not immunosuppressive for T-cells and macrophages; and (b) hypercholesterolemia per se was not the major determinant in the immunosuppression seen in studies dealing with lipids and immune function.

In most studies, marked hypercholesterolemia is induced by feeding diets high in fat with added and varying amounts of cholesterol and cholic acid, the hypercholesterolemic diet.

When such diets are fed, regardless of the quality of dietary fat, hypercholesterolemia is produced (11). Rats, in which serum cholesterol levels are normally in the 90- to 100-mg/100 ml range on most commercial chows, develop serum cholesterol levels of 350 to 500 mg/100 ml when fed diets containing cholesterol and cholic acid. However, depending on the quality of fat and as Broitman et al. (11) demonstrated, T-cell responsiveness to mitogen may be significantly depressed by a factor of 10 or more in hypercholesterolemic rats fed unsaturated fat compared to equally hypercholesterolemic rats fed saturated fat. Omitting dietary cholesterol and cholic acid from the diet decreased the serum cholesterol levels to approximately 100 mg/100 ml in groups of rats fed either saturated (20% coconut oil) or unsaturated fat (20% safflower oil), but the marked difference in T-cell responsiveness was maintained between the 2 dietary fat-fed groups (11).

There appears to be a consensus among some of our colleagues in immunology that the NK cell is of prime importance in immunosurveillance and neoplasia, although the complexities of the immune surveillance system would suggest that other cell types may be involved. In any case, there is no evidence that either saturated fat, unsaturated fat, or hypercholesterolemia affect NK cells. In our laboratory, NK cell
activity in rats fed diets containing either 20% coconut oil or 20% safflower oil, with or without added cholesterol and cholic acid, was the same (Chart 1). In addition, similarly treated animals do not lose their ability to reject syngeneic or allogeneic bone marrow transplants, a function presently attributed to M cells or NK cells such as iron deficiency which abrogates resistance to the growth of syngeneic bone marrow transplants (53) and which also suppresses T-cell function and promotes bone marrow transplants, a function presently attributed to M cells or NK cells (53).

There are nutritional manipulations which appear to affect M cells or NK cells such as iron deficiency which abrogates resistance to the growth of syngeneic bone marrow transplants (53) and which also suppresses T-cell function and promotes chemically induced carcinogenesis (65). Here, we would again remind the reader that every nutritional insult that promotes cancer has been shown to suppress some component of cell-mediated immunity (82, 63).

Dietary fat, like dietary protein, derives its biological value from its component parts. It seems reasonable and perhaps safe at this juncture to suggest that the effects of dietary fat on immune function are mediated through its component parts, the unsaturated fatty acids, specifically linoleic, linolenic, and arachidonic. One line of evidence suggests that cholesterol and unsaturated fatty acids exert their effect on immune cells by altering the lipid component of the cell membrane (18, 20, 38, 47, 56, 57). When lymphocytes are exposed to mitogen, there are marked alterations in the membrane phospholipid, fatty acid, and cholesterol composition (27–29, 38, 57). The addition of substrates which inhibit cholesterol synthesis to in vitro systems results in a decreased response of T-lymphocytes to mitogens (26, 38) and to decreased activity of cytolytic (killer) T-cells (26). Linoleic and arachidonic acids, as well as prostaglandins, inhibit T-cell responses to mitogen as does 25-hydroxycholesterol (7, 38), the latter being a potent inhibitor of cholesterol synthesis. The addition of cholesterol to these in vitro systems reverses the effect of 25-hydroxycholesterol (26) and enhances the response of T-cells to mitogen (29). The effects of 25-hydroxycholesterol, unsaturated fatty acids or prostaglandins do not appear to be the result of toxicity to the lymphocyte (45). However, the exact mechanism by which these substrates affect T-cell function is not clear, and several possibilities for the observed effects that have been discussed (12, 14, 20, 21, 27, 38) are: (a) an alteration in the fluidity of the membrane which may alter the configuration of receptor sites on the lymphocyte rendering it less competent or responsive; (b) alterations in cyclic adenosine 3′,5′-monophosphate concentrations or activation affecting antigen-lymphocyte interaction; (c) decreased macrophage function which would also affect antigen-lymphocyte interaction; and (d) decreased cholesterol synthesis within the cell which appears to be a prerequisite for DNA synthesis and subsequent blastogenesis (19).

When the fluidity of lymphocyte membranes is decreased in the presence of cholesterol, enhanced responses of T-cells to mitogen (29, 38) and increased cytolytic (killer) T-cell activity (26) occur. Membranes having a lower ratio of polyunsaturated to saturated fatty acids are generally less fluid than those having a higher ratio (38), ratios which can be affected by the quality of fat fed.

There appears to be a dichotomy. On the one hand, in in vitro studies, the addition of liposomal cholesterol to the media enhances T-cell responsiveness and overcomes the depressing effect of 7-ketocholesterol (29) and 25-hydroxycholesterol (26), both of which block cholesterol synthesis. Chen et al. (19) have demonstrated that cholesterol synthesis, which must and does precede DNA synthesis, is required for the generation of new membranes during blastogenesis. However, the addition of cholesterol liposomes would be expected to turn off cholesterol synthesis within the cell, according to feedback mechanisms outlined by Brown and Goldstein (13, 14), and therefore decrease T-cell responsiveness, which it does not in vitro. Obviously, membrane fluidity alone is not the controlling factor in blastogenesis nor are serum cholesterol levels or cholesterol synthesis per se. Rather, the gradients between medium, membrane, and intracellular cholesterol content (and synthesis) appear to be important determinants for optimal blastogenesis as suggested by several studies (26, 29, 38, 59). Since the quality of fat influences serum and cellular cholesterol levels, the effects of fatty acid on immune function and on oncogenesis may be mediated through changes in cholesterol metabolism or vis à vis prostaglandins.

Recently, Meade and Mertin (38) published an excellent review in which they concluded, "We have described the effects of fatty acids on immune cells in vitro and in vivo, but it is clear we are still largely at the stage of describing phenomena rather than understanding them. We may summarize by saying that a wide variety of fatty acids produce effects on both the lymphoid and the reticuloendothelial systems, the actual effects produced depending on the method of administration as well as the chemical nature of the fatty acid. With regard to fatty acids, diet, and disease, we consider it too early yet to say whether a role for fatty acids in the biochemistry of immune cells, or effects of fatty acids in vivo, have any relevance to human disease." We disagree with this conclusion. Most certainly dietary fat, and therefore fatty acids, plays some role in at least 2 major

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Chart 1. Effect of dietary saturated and unsaturated fat, with and without added cholesterol (1%) and cholic acid (0.3%), NK cell activity. Data are presented as log percentage of specific release ([lysis in presence of effector cells — lysis in the absence of effector cells]/[total possible lysis — lysis in the absence of effector cells]) plotted against log of the effector cell/target cell ratio (e/t).

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<thead>
<tr>
<th>Diet</th>
<th>NK Cell Activity</th>
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<tbody>
<tr>
<td>Diet I (Polyunsaturated Fat &amp; Cholesterol)</td>
<td>3.1 12.5 50 200</td>
</tr>
<tr>
<td>Diet II (Polyunsaturated Fat &amp; Cholesterol)</td>
<td>3.1 12.5 50 200</td>
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<tr>
<td>Diet III (Saturated Fat)</td>
<td>3.1 12.5 50 200</td>
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<tr>
<td>Diet IV (Saturated Fat &amp; Cholesterol)</td>
<td>3.1 12.5 50 200</td>
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\log \text{percent specific release} = \frac{\text{lysis in presence of effector cells — lysis in the absence of effector cells}}{\text{total possible lysis — lysis in the absence of effector cells}} \times \log \text{effector cell/target cell ratio (e/t).}
\]
diseases, cancer and atherosclerosis. It may not be clear as to how fatty acids exert their effects on immune function or to what extent one can transfer in vitro data to in vivo happenings. One cannot, however, completely ignore the overwhelming data, both experimental and epidemiological, that equate (a) dietary unsaturated fats with immunosuppression and promotion of various cancers, (b) increased dietary fat intake with an increased incidence of colon cancer and perhaps cancer at other sites, and (c) hypercholesterolemia and highly saturated fat diets with cardiovascular disease and atherosclerosis. Some readers may well ask about the intrusion of cardiovascular disease in this discussion on lipids and immunology. Several studies suggest an immune component in the pathogenesis of atherosclerosis (3, 27, 28). Alving et al. (3) have demonstrated that human complement can be activated in the presence of high concentrations of membrane cholesterol resulting in membrane change. These observations may have important implications clinically as well as in our understanding of the pathogenesis of atherosclerosis.

An association has been made between cardiovascular disease and colon cancer (56). Yet, there is no evidence from either experimental animals or from observations in humans that cardiovascular disease is associated with an increased risk of cancer or that the latter is associated with increased risk of the former. Both are associated with levels of dietary fat (11) or perhaps more importantly with the quality and quantity of fatty acids (11).

Indeed, all available experimental data would suggest that diets high in saturated fats would favor atherosclerosis whereas unsaturated fatty acids diets would favor neoplasia (2, 11) and that both affect some component(s) of the immune system which in turn probably enhances or promotes the disease process. If fats or unsaturated fatty acids play a role in oncogenesis and atherosclerosis, perhaps the roles are mediated through changes in or shifts between LDL and HDL. It is now generally accepted that high LDL levels, rather than cholesterol levels per se, are associated with an increased risk to atherosclerosis whereas high HDL levels are associated with a decreased risk to cardiovascular disease and that diet, smoking, alcohol, exercise, among other factors, can alter lipoprotein concentrations (64). What is of interest here is that serum lipoproteins have a regulatory role in immune responses presumably by regulating the expression of specific cell receptors (12, 14, 27, 28). Morse et al. (46) have demonstrated that lipoproteins isolated from normal human plasma inhibited the proliferation of lymphocytes stimulated by allogeneic cells or lectins, but to varying degrees. Intermediate LDL was the most potent inhibitor followed by very-low-density lipoprotein, LDL, and HDL. As is known, the binding of normal serum LDL by human fibroblast cells and lymphocytes influences the sterol content of these cells by modulating the rates of uptake, esterification, and synthesis of cholesterol (23, 27). At this juncture, it does not seem unreasonable to believe that lipoproteins play a role not only in the pathogenesis of atherosclerosis but also in at least some forms of cancer (48); that immune or host defense cells are affected in both diseases and that cholesterol concentrations per se may be uninformative about an individual’s risk to cancer, atherosclerosis or perhaps to other diseases. After all, cholesterol is an essential component of every cell, and normally its concentration is regulated to some degree. Finally, we offer one plausible explanation for the association between lipids and cancer. If tumor cells or transformed cells (premalignant) are no longer regulated in terms of cholesterol uptake, esterification, and synthesis, as has been shown (58), then changes in the concentration of LDL or HDL may enhance either the growth or the transformation of aberrant cells to malignant cells by further deregulating cholesterol metabolism. However, what seems like a “chicken and the egg” situation is not in fact. Since all of the experimental data suggest that no dietary manipulation in the absence of a carcinogen initiates cancer, then one can only assume that alterations in lipid metabolism influenced by at least the quality and quantity of dietary fat further perpetuates the defect(s) initiated by the carcinogen. Those same factors which promote carcinogenesis may in addition suppress those systems that normally destroy or keep aberrant cells in check. The tumor cell itself may further perpetuate itself by influencing lipid-cholesterol-regulating mechanisms of the normal cell. Perhaps it would be trite to state the obvious; more work must be done. George Mann may indeed be correct in his assessment (36); and possibly a new era is beginning with interest in the interactions of nutrition-immunology-heart-cancer.

References

25. DiLuzio, N. R., and Riggi, S. J. The development of a lipid emulsion for the
22. Committee of Principal Investigators. A cooperative trial In the primary
21 . Chisari, F. V., and Edgington, T. S. Lymphocyte E rosette inhibitory factor:
27. Ho, V. K., Brown, M. S., Bllheimer, D. W., and Goldstein, J. L. Regulation of
26. Helnlger, H.-J. , Brunner, K. T., and Cerottini, J.-C. Cholesterol is a critical
28. Ho, V. K., Brown, M. S., Kayden, H. J., and Goldstein, J. L. Binding,
30. Hughes, 0., Caspary, E. A., and Wisniewski, H. M. Immunosuppresslon by
32. Kos, W. L., Loria, R. M., Snodgrass, M. J., Cohen, D., Thorpe, T. G., and
33. Kumar, V. Diseases of immunity. In: S. L. Robbins and R. S. Cotran (eds.),
35. Lorla, A. M., Kibrlck, S., and Madge, G. E. Infection of hypercholesterolemic
37i0 CANCERRESEARCH VOL. 41
34. Loria, R. M., Kibrlck, 5., Downing, D., Madge, G. E., and Fllllos, L. C. Effects
41 . Mertln, J. Effect of polyunsaturated fatty acids on skin allograft survival and
42. Mertin, J., and Hughes, D. Specific inhibitory action of polyunsaturated fatty
43. Mertin, J., Hughes, D., Shenton, B. K., and Dickinson, J. P. In vitro inhibition
44. Mertin, J., and Hunt, R. Influence of polyunsaturated fatty acids on survival
46. Morse, J. H., Witte, L. D., and Goodman, D. S. Inhibition of lymphocyte
48. Nydegger, U. E., and Butler, R. E. Serum lipoprotein levels In patients
49. Offner, H., and Glausen, J. Inhibition of lymphocyte responses to stimulants
50. Pearce, M. L, and Dayton, S. Incidence of cancer in men on a diet high In
52. Ring, J., Selfert, J., Martin, J., and Brendel, W. Prolongatlon of skin allografts
53. Rodday, P., Bennett, M., and Vitale, J. J. Delayed erythropoiesis in irradiated
55. Rose, G., Blackburn, H., Keys, A., Taylor, H. L., Kannell, W. B., Paul, O.,
57. Shinitzky, M., and Inbar, M. Differenceln microviscosityinduced by different
58. Siperstein, M. D. Regulation of cholesterol biosynthesis In normal and
59. Tsoyoshima, 5., and Osawa, T. Cholesterol Inhibition of the temporary In
60. Toyoshima, S., and Osawa, T. Cholesterol Inhibition of the temporary In
62. Trudinger, P. J., and Doherty, P. C. The effect of calcium on the binding of
65. Vitale, J. J., Broitman, S. A., and Gottlieb, L. S. The effects of iron deficiency and the quality and quantity of
66. Warren, R. H., Roberts, D. W., and Blackburn, H. L. The effects of iron deficiency and the quality and quantity of
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