Tumor Biol 1988;9:225–232

1010–4283/88/0095–0225$2.75/0

Modulation of Cell-Mediated Immune Response by Steroids and Free Fatty Acids in AIDS Patients: A Critical Survey

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Key Words. Steroids · Fatty acids · Immune response · AIDS

Abstract. The overall data presented in this review show that cortisol and free fatty acids, in particular long-chain polyunsaturated fatty acids, each have immunoinhibitory properties on lymphoblastic transformation of certain T lymphocytes. This effect is enhanced when the two factors are associated. These data could explain in part the immunosuppression observed in acquired immunodeficiency syndrome (AIDS) patients where enhanced concentrations of cortisol and polyunsaturated fatty acids have been observed. The mechanisms which relate the action of the human immunodeficiency virus to the disturbance of steroidal hormonemia and lipid metabolism are discussed. The knowledge of these mechanisms would lead to new therapeutic measures against immunosuppression. These new weapons could be the administration of diets or treatments (liposomes) modifying the lipid profile of circulating cells and/or viruses and the utilization of hormonal therapy in AIDS and in some types of cancer which often present a biologic picture similar to that of AIDS.

Introduction

Long before the appearance of the acquired immunodeficiency syndrome (AIDS), numerous studies were undertaken on humoral and cell-mediated immunodeficiency. This was mainly investigated during pregnancy [1, 2], in order to explain the nonrejection of the fetus by the mother, during development [3, 4] and ageing [5], in leukemia [6] and during the course of immunosuppressive treatments [7–9]. The causes and mechanisms of these immunodeficiencies are far from being completely understood. The goal was, however, important, even before AIDS appeared, since a deficiency in the cellular immune surveillance is one of the factors associated with the appearance of cancer, especially during ageing when surveillance is frequently reduced [5].

The recent development in man of an infection by the human immunodeficiency virus (HIV), leading in a high percentage of contaminated subjects to a syndrome of severe and fatal cellular immunodeficiency, constitutes a new model for a better understanding of the molecular mechanisms involved during the appearance of immunodeficiency.

Most of the studies undertaken since the appearance of this syndrome in man have been largely oriented toward the identification of the virus, considered to be the only cause of the disease. As a matter of fact, many data, both epidemiologic (rate of progression, modes of contamination) and clinical (latent period, polymorphism of symptoms), challenge us as regards the existence of other factors required for the development of the disease [10]. In particular, the factors which determine the crossing of the frontier between healthy carriers or AIDS-related complex (ARC) and AIDS remain unknown.

Recent work has underscored the possible role of steroids, especially the glucocorticosteroids, and lipids, especially free fatty acids, in the evolution of the disease [11–14]. These two factors could act jointly, both on the infectivity of the virus as well as on the viability of lymphocytes, especially those whose functions are impaired during AIDS.

Role of Steroid Hormones on the Immune Response during AIDS

As AIDS sets in, clinical (anorexia, nausea, vomiting, loss of weight, diarrhea, fever and asthenia) and biologic (hyponatremia) signs suggestive of adrenal insufficiency appear [15–18]. In fact, in spite of a few histologic observations of lesions in the adrenal cortex, often due to local cytomegalovirus infection [18] or to drug toxicity [19], such lesions are not enough to explain the adrenal insufficiency [15, 17]. The latter could be a terminal-phase phenomenon, as it is in many other serious infections. Moreover, clinical data contrast with the results of static and dynamic adrenal function tests [17]. Cortisolemia at 8 a.m. was found to be significantly increased in ARC, and especially in AIDS at all stages of the disease [14, 15, 17, 20]. The other corticosteroids were found to be produced in subnormal concentrations [17]. The short-term (60 min) stimulation test with adrenocorticotropic hormone (ACTH) showed a subnormal response for cortisol and a clear decrease of 18-hydroxydesoxycorticosterone in AIDS [17]. Long-term (3 days) stimulation with ACTH brought to light a significant deficiency in mineralocorticosteroids [17]. This perturbation is less important and not always observed in ARC [17]. This finding could explain the hyponatremia very often present in AIDS patients. Thus, the appearance of this hormonal deficiency selectively in an ARC patient would indicate, were it to be confirmed, the passage of the disease to the stage of AIDS. It was recently observed that some patients with ARC or AIDS have a perturbed nycthemeral rhythm of cortisol secretion [20]. Moreover, patients in whom the number of CD4 lymphocytes is $< 100/mm^3$ have high cortisol levels at midnight. These interesting results should be confirmed in a larger number of cases. Taken together, these results raise the following questions.

(1) Is there a defect in the receptivity toward cortisol of certain tissues which could explain the clinical signs of adrenal insufficiency contrasting with the increase,

no doubt moderate but significant, of cortisol? (2) How can the raised cortisolemia of these patients be explained in view of the subnormal response of the adrenal to stimulation by ACTH? (3) Could there be a decrease in the catabolism of glucocorticosteroids in these cases, as strongly suggested by the findings of Klein et al. [11–13]? Recent work indicates that these results, obtained in vitro, would also be obtained in vivo, since a distinctly lowered concentration of 17-hydroxycorticosteroids, urinary metabolites of the glucocorticoids, was found in patients with ARC and AIDS in whom cortisolemia was above normal and free urinary cortisol normal or increased [14]. The mechanisms proposed by Klein et al. [11–13] in order to explain this diminished catabolism of cortisol will be discussed further. Lastly, these findings suggest the key question: what are the repercussions of the hypercortisolemia in patients who are HIV-positive on the viability of helper lymphocytes (CD4)? The blood levels of cortisol in these patients are suprapysiologic, but they are not as high as those observed in Cushing's syndrome. It is well known, on the other hand, that human lymphocytes are particularly resistant to glucocorticosteroids [6]. In fact, the mechanism by which glucocorticoids exert their immunomodulatory role is particularly complex [6, 7, 21, 22]. The mechanisms of action on both types of immunocompetent cells concerned in immunity, humoral and cellular, are diverse and for the most part necessitate cellular receptors for glucocorticosteroids [8, 21]. Moreover, the latter are not the only steroid hormones implicated. Immunocompetent cells are influenced, positively or negatively, by different steroids [23] like progesterone [24], glucocorticoids [22, 25], androgens [26, 27] and estrogens [28, 29]. It would therefore be necessary to study the variations of steroids other than the corticosteroids in patients with AIDS at different stages of the disease. Contrariwise, characteristic modifications of glucocorticoids are observed during the immune response [30, 31]. The significance of such a network of immunoendocrine interactions, with afferent and efferent pathways, is slowly beginning to emerge [30]. It corresponds to the triggering, when contact with antigen takes place, of a mechanism which mobilizes, selectively and in a temporally programmed manner, one or a few lymphocyte clones responding specifically to the antigenic challenge. A normal immune response implies the elimination or neutralization of other clones which could have been recruited by a bystander effect. Thus, the introduction of antigen in an organism stimulates antibody formation, but also provokes the secretion of various steroids, including glucocorticoids. The latter might function to block other lymphocytes such as those which could be recruited, for example, by lymphokines produced by stimulated monocytes. A similar mechanism could explain why, when two antigens are injected simultaneously or, more often, sequentially, the immune response to the second is feebler than that observed when it is injected alone. Adrenalectomy prior to the injection of two antigens leads to a good immune response to both of them [30].

Furthermore, studies by Galili et al. [6] have shown that certain lymphocytes, activated in different pathologic conditions, are lysed by physiologic concentrations of cortisol. These studies [6, 30] have an important bearing on the repercussion on immunocompetent cells infected by HIV of the higher permeation of AIDS patients with cortisol.

Lipid Variations and Immune Response in AIDS

The lipid environment of cells, and, in particular, of circulating immunocompetent cells, conditions the lipid structure of their plasma membrane, and through that most likely, their function. Many studies have shown that lipids influence the activity of macrophages and lymphocytes [32]. For the latter, it has generally been found that mitogens provoke the liberation of free fatty acids, which in term, especially unsaturated fatty acids, inhibit the lymphoblastic response induced by mitogens. Furthermore, it has been shown that the increased serum free fatty acids in patients with acute lymphocytic leukemia brought about the inhibition of the lymphoblastic transformation provoked by mitogens in normal lymphocytes [33].

This inhibition could be brought about by different mechanisms, the main ones being related to the modification of the lipid structure of the lymphocyte membrane, and thereby of the function of receptors and enzymes intercalated in the lipid bilayer, and to a possible imbalance of the oxidoreductive potential of the cell in favor of oxidation of polyunsaturated fatty acids and/or the formation of prostaglandins [32, 33].

On one hand, in ARC and AIDS patients, at the beginning of infection, the percentage of free polyunsaturated fatty acids in serum is double that of controls, whilst that of oleic acids is very significantly reduced and the concentration of total free fatty acids does not vary. Thus, the ratio stearic acid/oleic acid (C18:0/C18:1) is increased [14].

On the other hand, the ratio stearic acid/oleic acid of red and white blood cells of not only AIDS but also cancer patients is very notably reduced due to an increase in oleic acid in the membranes of these cells [34].

It can be hypothesized that a preferential incorporation of oleic acid into lymphocyte membrane phospholipids occurs in AIDS patients. It should be recalled that normally activated lymphocytes preferentially incorporate arachidonic acid [32, 33]. This polyunsaturated acid, when it is not incorporated, could be free in the plasma and be transformed either to prostaglandins or leukotrienes, and/or peroxide derivatives which can modify the activity of certain enzymes, membrane receptors or nuclear proteins, and even nucleic acids. These modifications of lipid membranes could facilitate the formation of syncytia, which is what is observed in AIDS patients [34]. Fatty acids of lipids of cells with syncytial transformation following infection with a virus have a lowered C18:0/C18:1 ratio [34].

Moreover, the membrane conformation, conditioned by the lipid structure of the lymphocyte on one hand, and of HIV on the other hand (the virus also having a lipid bilayer structure [35]), should be such that the two proteins, gp 120 of the virus and the CD4 antigen of the lymphocyte, can associate for infection to occur [36]. It is conceivable that a dietary regimen leading to the modification of the lipid structure of one of these two elements could favor, or hamper, the propagation of the virus. It also appears that the use of an antioxidant such as butylated hydroxytoluene can lessen the virulence of HIV in vitro [35]. Were this to be confirmed, it would underscore the importance of oxidative phenomena, especially those of unsaturated fatty acids, in the physiopathology of AIDS.

Effect of Steroid Hormones on Membrane Lipid Structure and Effect of Fatty Acids on Glucocorticoid Metabolism

It has been shown above that the lipid structure of membranes conditions the lymphocyte response and the infectivity of the virus. The lipid structure depends on nutritional and hormonal factors, in particular, the acyltransferases, the activity of which conditions the incorporation of fatty acids into membrane lipids, and the lipases (phospholipases, triglyceride lipase, lipoprotein lipase), which liberate free fatty acids. Those which are incorporated into phospholipids are under the control (biosynthesis or activity of the enzyme) of numerous factors, amongst which are the steroid hormones (progesterone, DHEA sulfate, glucocorticoids, estrogens [37–41]). A mechanism in equilibrium of incorporation-liberation prevails, which depends, respectively, on an acyltransferase and a lipase. This mechanism is strictly controlled. In the uterus, estradiol activates it, whereas progesterone inhibits it [39]. This balance is disturbed in AIDS as we have seen. The perturbation could be due to modifications in the nature and the concentration of steroid hormones which maintain normal equilibrium. At the lymphocyte level, glucocorticoids would intervene in the following order: glucocorticoids→lipase activation→liberation of free fatty acids (oxidation?)→lymphocytolysis.

A direct action of the virus on the enzymes controlling the lipid structure of the membrane cannot be excluded. Further knowledge of the hormonal and/or viral control in AIDS of acyltransferase and lipase functions would lead to a better understanding of this mechanism. These enzymes could, under different conditions which remain to be established, favor the incorporation into phospholipids of one type of fatty acid rather than another.

As stated earlier, cellular lipids of blood cells of AIDS and cancer patients contain increased amounts of oleic acid and possibly less arachidonic acid than those of normal subjects [34]. These anomalies could be caused by an imbalance in the function of the aforementioned enzymes.

Furthermore, studies by Klein et al. [42] have shown that ethanol extracts of sera of AIDS patients, as well as of patients with cancer of the colon and cirrhosis of the liver, and also sera of the newborn have the property of reducing lymphocyte viability in the presence of physiologic concentrations of cortisol [42]. This effect can also be obtained, at least partially, when an unsaturated fatty acid, e.g. linoleic acid, is used in place of the ethanol extract [13]. Addition of a lipase to the ethanol extract of sera of cancer patients enhances the effect on cell viability, whilst the addition of albumin inhibits it, at least partly [43]. The possibility that unsaturated fatty acids are transformed into more active oxigenated derivatives is not excluded and should be verified. These workers have also shown that this extract causes inhibition of the catabolism of cortisol by lymphocytes [13, 14]. The latter then become, like thymocytes, very vulnerable to this hormone.

Figure 1 summarizes these new data. It also takes into account the fact that unsaturated fatty acids can selectively favor, according to their concentration, the liberation of progesterone, or of cortisol, from their common binding protein, the corticosteroid-binding protein [44]. The concentration of free cortisol or progesterone, the only active and metabolizable form of the hormones, therefore depends on the concentration of free unsatu-

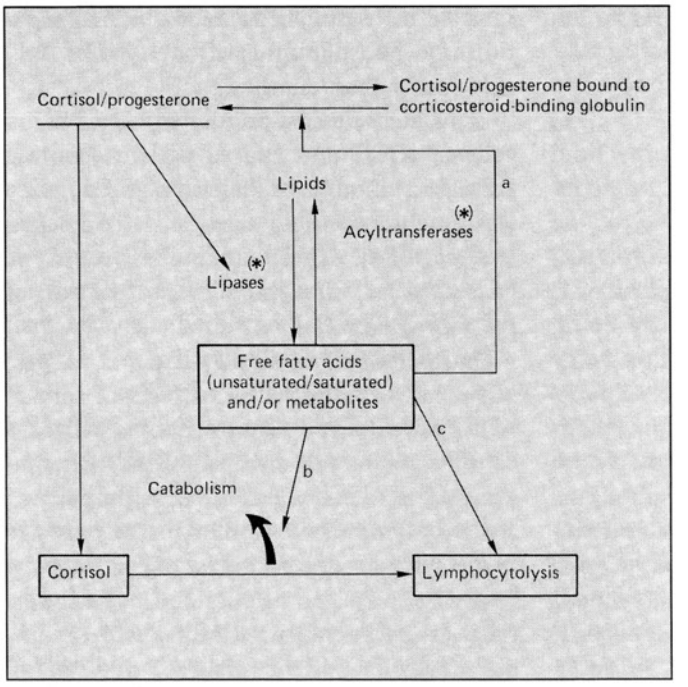

Fig. 1. Saturated and unsaturated fatty acids liberated from circulating or membrane lipids after the action of steroid-dependent (*) (progesterone, corticoids, androgens and estrogens) lipases and acyltransferases could intervene at three levels: **a** by modifying the equilibrium of the binding between protein of transport (corticosteroid-binding globulin, shown here, but also sex steroid binding protein on which estradiol, testosterone and dihydrotestosterone are bound) and the steroids fixed on them; **b** by inhibiting the catabolism of cortisol by lymphocytes, and **c** by modifying the lipid structure of the lymphocyte membrane and thereby modifying the function of receptors (for mitogens, antigens, HIV) and membrane enzymes involved in the transfer of the mitogenic information. Fatty acids thus participate in the process of lymphocytolysis.

rated fatty acids present in the medium. The concentration of saturated fatty acids could also play a role. It has thus been observed that the effect of unsaturated fatty acids could be minimized by saturated fatty acids [32, 45] and that saturated fatty acids can, in some cases, induce lymphocytolytic activity [46].

In conclusion, the overall data presented in this review demonstrate that cortisol and unsaturated free fatty acids each have immunoinhibitory properties and that the latter are enhanced when the two are associated. These new data could explain how in some pathophysiologic situations, which can be compared to those described in physiologic situations [6, 30], glucocorticoids inhibit lymphoblastic transformation of certain T lymphocytes. The inhibitory effect observed at physiologic concentrations of glucocorticoids could be due to the presence of high concentrations of free unsaturated, and, sometimes, saturated fatty acids. It would be of moment to continue this work, with a view to understanding, in the syndrome of immunodeficiency characteristic of AIDS and of cancer, the mechanisms which relate the action of HIV or of oncogen to the disturbances of steroidal hormonemia and lipid metabolism. It would be especially interesting to find out why CD4 lymphocytes are particularly sensitive to these hormonolipidic factors.

The knowledge of these mechanisms would lead to new therapeutic measures against immunosuppression. These new weapons could be the administration of diets or treatments (liposomes [35]) modifying the lipid profile of circulating cells and/or viruses, and the utilization of hormonal therapy in AIDS and in some types of cancer which often present a biologic picture akin to

that of AIDS. These studies must be undertaken keeping in mind that free fatty acids or hormones can exert opposite effects, depending on their nature and concentration, on the multiplication of immunocompetent and cancerous cells.

Acknowledgments

This work was supported by grants from UER Xavier-Bichat (University Paris VII), 'Fondation pour la recherche médicale', 'Ligue française contre le cancer', and INSERM AIDS program.

The authors are grateful to P. Neant for her excellent secretarial assistance and Dr. S. Gauthier-Rahman for help in preparing the manuscript.

This review includes the research undertaken in the INSERM Unit 224, Biochemistry Laboratory, X.-Bichat Hospital, and Infectious Pathology Department (Prof. J.P. Coulaud), Claude-Bernard Hospital Paris, by N. Christeff, C. Michon, G. Goertz and J. Hassid [14].

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Accepted: May 2, 1988

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