for both PHA-induced transformation and mixed lymphocyte reactivity was found almost entirely in the third peak of a G-200 eluate.

It is of particular interest to determine whether the inhibitory capacity of mouse serum varies with changes in the immunological status of the donors. Sera were obtained from SW mice injected with live BCG vaccine, which causes proliferation of macrophages[7]. The effects of sera obtained after a single injection were variable, but the depressive effect was generally not greater than that of normal sera. As shown in Table II, sera obtained from mice 2 weeks after the second of 2 injections of BCG were substantially more depressive than normal sera. This difference was scarcely apparent 6 weeks later.

Sera were also obtained from SW mice treated with antilymphocyte globulin (ALG) which causes, among a variety of biological effects[8], a temporary depletion of T lymphocytes[9] and may cause some proliferation of macrophages[10]. They received 3 s.c. injections, at daily intervals, of 0.5 mg of a preparation shown to be immunosuppressive[11]. The sera had an increased depressive capacity up to 6 days after the first injection (mean thymidine incorporation 56% of that in normal mouse serum). A second wave of increase in depressive capacity occurred later, maximal at 18 days (mean thymidine incorporation 35% of that in normal mouse serum).

Nude mice (genetically *nu/nu*) lack T lymphocytes[12] and cannot make cell-mediated immune response[13]. Sera from such mice were compared with sera from heterozygous (*nu/+*) siblings, at concentrations from 1.25 to 5% in the presence of 2.5% human serum and optimal or $^1/_4$ optimal doses of PHA with spleen cells from *nu/+* mice. Both sets of sera had a pronounced depressive capacity (67% for *nu/nu* serum and 63% for *nu/+* serum at 5% and with an optimal dose of PHA).

These findings indicate that normal mouse serum contains a factor which depresses the response of normal mouse spleen lymphocytes to PHA and to allogeneic lymphocytes. The factor appears to be a highly charged molecule of relatively low molecular weight. These properties make it highly unlikely that the factor is endotoxin[14] or an immunoglobulin. It is thus also unlikely that antibodies to PHA are responsible for inhibition of the response to PHA[15] and in any case mixed lymphocyte reactivity is also inhibited. The presence of depressive activity in sera from Nude mice and ALG-treated mice makes an origin from T lymphocytes very unlikely. Its possible origin from, e.g., bone-marrow derived (B) lymphocytes or macrophages is being investigated. Other current investigations concern the depressive effects of sera from mice subjected to more violent immunological insults, such as graft-versus-host reactions, and the effects of mouse sera on other activities of stimulated lymphcoytes, e.g., RNA and protein synthesis and cytotoxicity. The should shed light on the possible homeostatic role of the depressive factor and its relationship to the 'immunoregulatory α-globulin' isolated from some batches of normal human plasma[16] and the depressive factor(s) in normal chicken serum[17,18].

Résumé. Le sérum des souris normales contient un facteur, de haute charge négative et de faible poids moléculaire, qui abaisse la réponse de lymphocytes spléniques au PHA et aux lymphocytes allogéniques. Le sérum des souris qui ont eu une injection de BCG est même plus dépressif que le sérum des souris normales.

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Lymphocyte-Fibroblast Interaction in the Pathogenesis of Inflammatory Gingival Disease

Gingivitis is a destructive inflammatory disease of the tissues surrounding the teeth. It is extremely widely spread in human populations[1]. The disease is related etiologically to the accumulation and growth of microorganisms (specifically termed dental plaque) on the surface of the teeth[2]. The early gingival lesion is characterized by an infiltration of leucocytes and a concomitant reduction in the amounts of connective tissue substance. While the disease has been the object of intensive investigation, the nature of its pathogenesis has not been defined.

The data reported here were derived from an extensive series of studies using quantitative morphometric techniques for the analysis of human gingival biopsy specimens. The techniques used for preparation and analysis of the material are described in detail elsewhere[3] and a detailed report of the observations is being prepared[4]. The data show that a form of delayed hypersensitivity in which sensitized lymphoid cells appear to exert a cytotoxic effect on fibroblasts of the gingiva may play a major role in the induction and progress of the disease.

Volumetric data were obtained for the cell and fiber composition of the infiltrated connective tissue (ICT) and non-infiltrated connective tissue (NCT) of 20 human

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gingival biopsy specimens using standardized, random sampling electron microscopy. The biopsies stemmed from children 9 to 15 years of age, with clinical symptoms of slight to moderate gingivitis. Comparison of the volumetric density of collagen fibers per unit volume of ICT and NCT demonstrated a reduction of 70% in the fiber population of the ICT fraction. This observation agrees well with previous reports in which biochemical techniques were used[5].

The percent cell composition of the ICT exclusive of vascular structure was fibroblasts 14.8, neutrophilic granulocytes 2.6, monocytes and macrophages 2.1, small lymphocytes 39.3, medium lymphocytes 34.9, immunoblasts 1.9, plasma cells 2.0 and mast cells 2.4. It is noteworthy that significant numbers of immunoblasts were observed and that cells of the lymphoid series comprised 76.1% of the total cell population.

Specific cytopathic alterations were observed in the fibroblasts of the ICT fraction. The average size of these cells was increased 3-fold relative to the fibroblasts present in the NCT (Figure 1). Distinctive cytologic alterations observed in the fibroblasts included electron lucency of the nucleus suggestive of a reduced chromatin content, frequent absence of nucleoli, widely dilated cisternae of the rough endoplasmic reticulum, swollen mitochondria frequently with loss of cristae, and rupture

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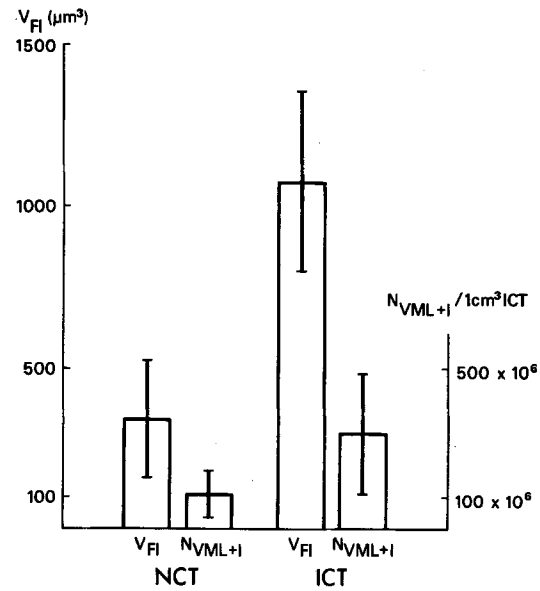

Fig. 1. Average (± standard deviation, vertical bars) volume of single fibroblasts (V_{Fl}) and average numerical density (N_V) of medium-size lymphocytes (ML) and immunoblasts (I) per 1 cm³ of tissue in normal (NCT) and infiltrated (ICT) connective tissue, measured in gingival biopsies from 20 individuals. For $N = 40$ (pool of NCT and ICT data pairs) the correlation coefficient ($r = +0.55$) is highly statistically significant.

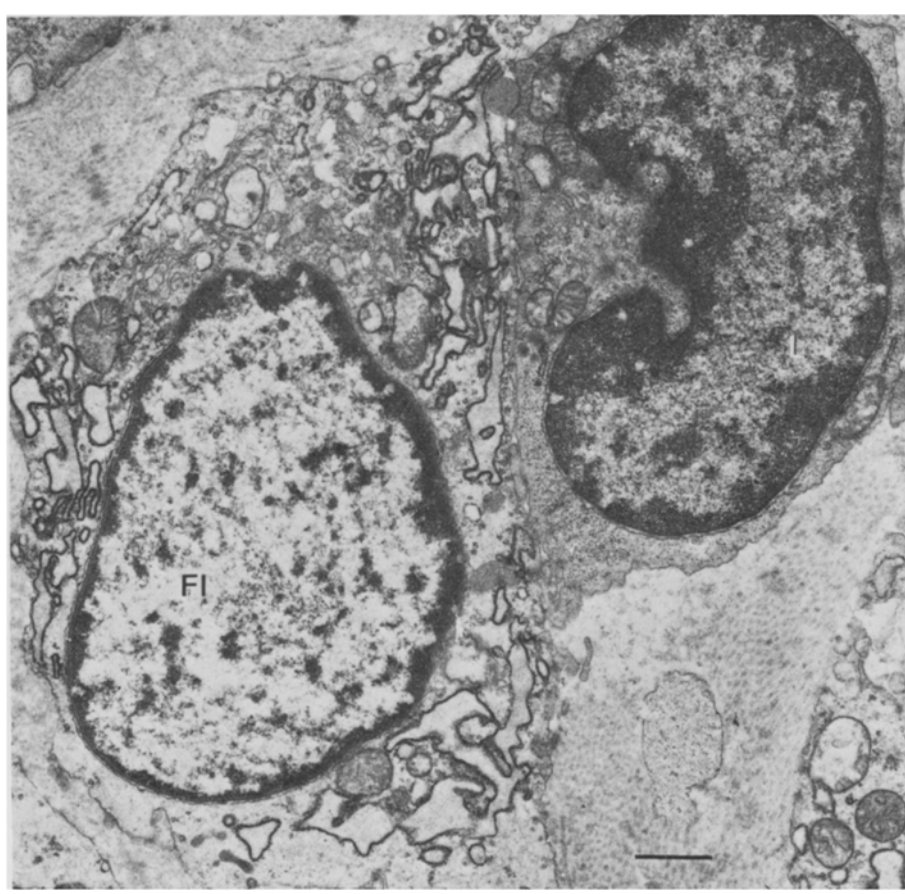

Fig. 2. Fibroblast (Fl) with closely neighbouring lymphocyte (L) residing in the infiltrated connective tissues fraction. Note nuclear electronlucency dilated cisternae of rough endoplasmic reticulum and mitrochondial alterations in fibroblast, and well preserved lymphocyte structures. Collagen fibrils are observed to surround both cells. ×13,800.

of the plasma membrane (Figure 2). These alterations are characteristically exhibited by sick or dying cells. The changes do not appear to be a consequence of defective tissue fixation and processing since they were not seen in fibroblasts or other cells of the NCT – nor in the non-fibroblastic cells of the ICT fraction.

The changes observed in the fibroblasts of the ICT appear to be associated with the activity of lymphoid cells. There is a strong positive correlation ($r = +0.55$) between the increasing fibroblast size and increasing numbers of medium-size lymphocytes and immunoblasts (Figure 1). Furthermore, lymphocytes were observed frequently in intimate contact with the altered fibroblasts in the ICT-fraction (Figure 2) while these associations were not observed in the NCT-fraction.

Recently it was shown that peripheral blood lymphocytes obtained from patients with inflammatory gingival disease are sensitized to antigenic substances present in human dental microbial plaque[6]. These cells undergo blast transformation when cultured in vitro in the presence of plaque antigens, and fluids from these cultures exert a marked cytotoxic effect on gingival fibroblasts[7]. The morphologic and morphometric data presented here support the idea that a phenomenon similar to that observed in vitro may be occurring in the gingival tissue in early gingival disease.

The observations provide a plausible basis for the early lesion in gingivitis. In susceptible individuals, lymphoid cells become sensitized to microbiologic substances present in dental plaque. Upon encountering these substances in the region of the dento-gingival area, the cells undergo blast transformation and interact directly or through mediators with resident fibroblasts to induce cytopathic alterations. The affected fibroblasts are no longer able to produce and maintain the connective tissue substance of the gingiva and this leads to the observed loss of connective tissue substance and loss of normal function.

Zusammenfassung. Eine morphometrische Analyse menschlicher, entzündeter Gingiva ergab im Vergleich zu normalem Bindegewebe 70% weniger Kollagenfasern, dreifach vergrösserte und pathologisch veränderte Fibroblasten und eine kleine Population charakteristischer Immunoblasten mit einer Zellansammlung, die zu 76% aus Zellen der Lymphozytenserie bestand. Die Grösse der Fibroblasten war positiv mit der steigenden Zahl der Lymphozyten korreliert. Diese Befunde weisen auf eine Immunreaktion mit zytotoxischen Auswirkungen auf Fibroblasten des gingivalen Bindegewebes hin.

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Antiphlogistic Activity of L-Phenylalanine and L-Tryptophane within the Mouse Peritoneal Cavity

Since the administration of irritants into the abdominal cavity produces a predictable inflammatory response characterized by an increased % polymorphonuclear leukocytes (PMN) I have attempted to inhibit the infiltration of PMNs into an inflamed peritoneal cavity induced by an intraperitoneal injection of gelatin solution by injecting L-phenylalanine and L-tryptophane[1]. MCGOWAN and I (1968) have found that in women with inflammation of the peritoneal cavity the PMN counts were elevated 2 or 3 times above normal[2]. The possibility that some amino acids have anti-inflammatory activity was suggested to us by the observation that certain amino acids maintain life and increase liver glycogen in adrenalectomized rats[3–5]. Both biological activities characterize adrenal steroids which have anti-inflammatory activity. Furthermore, we have shown that L-phenylalanine and L-tryptophane inhibited PMN infiltration into an area of local gelatin-induced inflammation under the skin of adrenalectomized rats[6] – a reliable procedure for testing the local antiphlogistic activity of glucocorticosteroids[7].

Adult female CF-1 mice (25–30 g) were given a single i.p. injection of aqueous 1% gelatin solution and we injected control animals with water. Aqueous L-phenylalanine and L-tryptophane were each administered i.p. at 25 mg/kg simultaneously but separately with gelatin. The 1% gelatin solution was injected on a 100 mg/kg per 10 ml basis whereas the 0.25% amino acid solutions were administered on the basis of 25 mg/kg/10 ml. The 10 ml volume per kg of body weight was employed for control animals. When the gelatin solution was injected simultaneously with the amino acid the total volume was maintained equal to the gelatin alone so that we would not obtain a response due to a gelatin dilution factor. In 3 h peritoneal fluid (sometimes only one drop) was aspirated and spread on an albumin-coated slide. Each coded slide was fixed in 95% alcohol and stained with buffered Wright's stain. 200 cells were consecutively and randomly counted so that we could determine the % distribution of lymphocytes, polymorphonuclear leukocytes, monocytes, eosinophils, other mononuclear cells and mast cells present in each cytologic specimen. This procedure of randomly counting a fixed number of cells rather than counting the number of cells in an absolute fluid volume eliminates the extreme variation resulting from counting cells per unit volumes because of the small amount of fluid aspirated. The average values reported represent mean % distribution of cells obtained

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