SOA production or on release of azurophil granule contents (lysozyme, elastase, and myeloperoxidase) or specific granule contents (lysozyme) (Table 1). Concentrations of nicotine up to 3 m*M* also failed to modify the effects of fMLP on SOA production or degranulation. Higher nicotine concentrations significantly reduced SOA and enzyme release but also reduced cell viability as estimated by trypan blue dye exclusion.

Our findings indicate that nicotine is chemotactic for PMN's. Unlike C5fr, fMLP, and a number of other factors chemotactic for PMN's, it does not enhance SOA generation or degranulation. In this respect, nicotine is similar to the peptide Gly-His-Gly (*8*) and to the chemotactic factor generated from plasma by SOA (*9*).

Our data also indicate that nicotine enhances PMN responsiveness to other chemotactic factors. Nicotine may thus alter receptor affinity for other chemoattractants or affect membrane fluidity, causing a faster start-up response to chemoattractants, as reported for *n*-propanol and *n*-butanol in combination with fMLP (*10*). The effects we observed may be mediated by noncholinergic nicotine receptors on PMN's (8.7×10^4 per cell) (*11*). This possibility deserves further study, but the described PMN nicotine receptor affinity for nicotine is 36 n*M*, while significant nicotine effects in our study were observed in the range 0.31 to 31 μ*M*. The differences between the chemotactic responses of PMN's and monocytes to nicotine suggest that monocytes do not have nicotine receptors.

Our results contrast with the inhibition by nicotine of the chemotactic response of PMN's to casein reported by Bridges *et al.* (*12*). Of the many differences between our experimental protocol and that of Bridges *et al.*, the most important appears to be that we used subcytotoxic rather than toxic concentrations of nicotine.

Since the optimum concentration for nicotine-induced PMN chemotaxis is approximately 100 times higher than that typically found in the plasma of cigarette smokers, nicotine might appear to be a negligible stimulus to the ingress of PMN's into the lungs of smokers. However, during smoking the intrapulmonary concentration of nicotine, especially in the bronchoalveolar lining fluid, may be much higher than the plasma concentration. Moreover, nicotine, even at concentrations found in the plasma of smokers, makes PMN's more responsive to other chemotactic factors in vitro. Nicotine may also enhance PMN recruitment in response to the tobacco smoke–induced release of the PMN chemotactic factor by alveolar macrophages (*6*). This possibility should be amenable to testing in vitro.

Nicotine is the most widely used addictive alkaloid in the world. Our results suggest that it may promote pulmonary inflammation and may thus have a prominent role among smoke components in the pathogenesis of lung injury in cigarette smokers.

NOEL TOTTI III*
KEVIN T. MCCUSKER
EDWARD J. CAMPBELL†
GAIL L. GRIFFIN
ROBERT M. SENIOR

Respiratory and Critical Care Division, Department of Medicine, Jewish Hospital of St. Louis, Washington University Medical Center, St. Louis, Missouri 63110

References and Notes

1. J. A. Califano, Jr., *DHEW Publ. (PHS) 79-50066* (1979).
2. D. E. Niewoehner, J. Kleinerman, D. B. Rice, *N. Engl. J. Med.* **291**, 755 (1974).
3. A. Janoff *et al.*, *Am. Rev. Respir. Dis.* **115**, 461 (1977); R. M. Senior *et al.*, *ibid.* **116**, 469 (1977).
4. K. H. Kilburn and W. McKenzie, *Science* **189**, 634 (1975); J. R. Hoidal and D. E. Niewoehner, *Am. Rev. Respir. Dis.* **126**, 548 (1982).
5. G. W. Hunninghake, J. E. Gadek, O. Kawanami, V. J. Ferrans, R. G. Crystal, *Am. J. Pathol.* **97**, 149 (1979).
6. G. Hunninghake, J. Gadek, R. Crystal, *Chest* **77**, 273 (1980); G. Hunninghake, J. E. Gadek, H. M. Fales, R. G. Crystal, *J. Clin. Invest.* **66**, 473 (1980).
7. M. A. Russell, M. J. Jarvis, G. Devitt, C. Feyerabend, *Br. Med. J.* **283**, 814 (1981).
8. I. Spilberg, B. Mandell, J. Mehta, T. Sullivan, L. Simchowitz, *J. Lab. Clin. Med.* **92**, 297 (1978).
9. W. F. Petrone *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1159 (1980).
10. I. Yuli, A. Tomonaga, R. Snyderman, *ibid.* **79**, 5906 (1982).
11. B. D. Davies, W. Hoss, J. P. Lin, F. Lionetti, *Mol. Cell. Biochem.* **44**, 23 (1982).
12. R. Bridges, J. H. Kraal, L. J. T. Huang, M. B. Chancellor, *Infect. Immun.* **16**, 240 (1977).
13. Polymorphonuclear neutrophils were freshly separated from heparinized venous blood of healthy nonsmokers by Ficoll-Hypaque centrifugation followed by dextran sedimentation [A. Boyum, *Scand. J. Clin. Lab. Invest.* **21**, 77 (1968)] and erythrocyte lysis. For details, see E. J. Campbell, R. M. Senior, J. A. McDonald, D. L. Cox, *J. Clin. Invest.* **70**, 845 (1982). Final PMN suspensions (2.5×10^6 cells per milliliter) were in Dulbecco's modified Eagle's medium supplemented with human serum albumin (1 mg/ml; American Red Cross Blood Services).
14. T. F. Deuel, R. M. Senior, J. S. Huang, G. L. Griffin, *J. Clin. Invest.* **69**, 1046 (1982). PMN's were placed on double membranes [a 2.0-μm-pore membrane (Nuclepore) overlying a 0.45-μm-pore membrane (Millipore)] that separated the upper and lower compartments. Cells that penetrated the upper membrane during the assay (incubation for 1 hour at 37°C in air with 5 percent CO_2) were counted. The nicotine was obtained from Eastman Kodak.
15. Suspensions of PMN's were incubated with various nicotine concentrations at 37°C in air with 5 percent CO_2 for 90 minutes, washed twice with fresh medium, and assayed for responsiveness to chemotactic peptides. The fMLP was from Sigma and the C5fr [J. Fantone, R. M. Senior, D. L. Kreutzer, M. Jones, P. A. Ward, *J. Lab. Clin. Med.* **93**, 17 (1979)] was from D. L. Kreutzer.
16. B. M. Babior, R. S. Kipnes, J. T. Curnutte, *J. Clin. Invest.* **52**, 741 (1973).
17. V. Massey, *Biochem. Biophys. Acta* **34**, 255 (1959).
18. E. F. Osserman and D. P. Lawlor, *J. Exp. Med.* **124**, 921 (1966).
19. *Worthington Enzyme Manual* (Worthington Biochemical, Freehold, Md., 1972), pp. 43–45.
20. D. R. Bielefeld, R. M. Senior, S. Y. Yu, *Biochem. Biophys. Res. Commun.* **67**, 1553 (1975).
21. This work was supported in part by PHS grants HL30341, HL16118, and HL29594 and by the Council for Tobacco Research, Inc. We thank M. A. Campbell for excellent technical assistance.
* Present address: Veterans Administration Hospital, Pulmonary Division, San Juan, Puerto Rico 00936.
† To whom correspondence should be addressed.
23 June 1983; accepted 20 September 1983

Heterogeneity of Normal Human Diploid Fibroblasts: Isolation and Characterization of One Phenotype

Abstract. *Cultures of human diploid fibroblasts contain cells that respond to exposure to the first component of complement (C1) by initiating DNA synthesis and growth. The plasma membranes of these cells have specific binding sites for the C1q subcomponent of C1. A fluorescence-activated cell sorter was used to isolate a subset of cells with a high affinity for C1q, and the growth and synthesis activities of these high-affinity cells were studied after numerous replications in vitro. These cells synthesize DNA and grow faster than the parent cultures and low-affinity cells, and they produce two to three times as much protein. About 40 percent of their total protein synthesis activity is directed to collagen production, unusually high proportions of collagen types III and V being produced. These properties and the high affinity of the cells for C1q are retained for at least six cell transfers. This phenotype has the properties expected of fibroblasts in healing wounds and inflamed tissues.*

Resident fibroblasts are responsible for the production and maintenance of the connective-tissue matrix and for repair after injury. The growth and synthesis activities of these cells are controlled by intrinsic genetic factors and by environmental ligands present in the tissue fluids such as epidermal growth factor, platelet-derived and fibroblast growth factors, complement proteins, prostaglandins, and factors released by lymphoid cells and macrophages (*1–3*).

These environmental ligands modulate cell activities by a concentration-dependent, reversible binding to specific sites on the surfaces of fibroblasts (*4*). However, a mechanism of this kind cannot account for the behavior of these cells in some aspects of growth and differentiation and in certain pathologic situations.

For example, cells obtained from the site of lesions in scleroderma, keloid, atherosclerosis, phenytoin-induced gingival hyperplasia, and periodontitis exhibit unusual phenotypic properties and these persist through numerous cell transfers in culture (*5, 6*).

We have suggested that, in addition to being modulated by growth factors and environmental ligands, fibroblasts are functionally heterogeneous, the composition of any given normal or diseased connective tissue being a consequence in part of its particular mixture of fibroblast subtypes, and that subtype deletion or amplification can result from selective cytotoxic and mitogenic responses induced by the binding of environmental ligands (*3, 6, 7*). Many investigators (*8*) have reported heterogeneity in morphology, synthesis activities, and proliferative potential among individual cells of mass cultures of human diploid fibroblasts. Although the heterogeneity hypothesis has been supported by the isolation of phenotypically unusual fibroblasts from diseased human connective tissue (*6*), this hypothesis has not been definitively proved.

In investigating the heterogeneity hypothesis, we reasoned that if distinct subtypes of fibroblasts exist they should be present in healing wounds and at sites of inflammation. At such sites, the subtype or clonal selection process could be initiated and perpetuated by factors such as the high molecular proteins of plasma, which do not normally have access to the extravascular compartment but which flood it during inflammation and following injury. The first component of complement (C1), a protein of approximately 774 kilodaltons is such a protein. We have shown that a subset of human diploid fibroblasts manifests specific binding sites for the collagenous portion of the C1q component of C1 (*9*) and that C1 induces a mitogenic response in this subset of cells (*2*). Using a fluorescence-activated cell sorter, we have isolated this subset of fibroblasts; we have partially characterized it and have shown that it gives rise to like progeny through several transfers in vitro. The cells manifest growth and synthesis properties expected of cells located at sites of inflammation or those participating in wound healing.

Table 1. Cell function characteristics of a human gingival fibroblast subtype. DNA synthesis was measured after a 4-hour treatment with [^{3}H]thymidine (*2*) and is reported as counts per minute per 10^3 cells. For the measurement of protein synthesis, cells in serum-free medium were labeled with [^{35}S]methionine for 20 minutes, then washed, and the amount of bound radioactivity was measured in a beta-counter (*10*). Data are reported as counts per minute per 10^3 cells. For the measurement of collagen synthesis, cells were labeled with [^{3}H]proline for 6 hours, and newly synthesized protein was harvested (*10*). Collagen synthesis was assessed by measuring collagenase-digestible radioactivity as disintegrations per minute per 10^3 cells. Cells and medium proteins labeled with [^{3}H]proline were digested with pepsin to convert procollagens to collagens, followed by separation on DEAE-cellulose at 15°C, with 0.11*M* NaCl used to elute collagen types I and III and 0.25*M* NaCl used to elute type V (*12*). Fractions were separated electrophoretically on 5 percent slab gels. Fluorograms were prepared, and collagen types were measured with a scanning densitometer (*11*). N.D., not determined.

Type of cell	DNA synthesis	Total protein synthesis	Collagen synthesis	Collagen type (%)		
				Type I	Type III	Type V
High-affinity	7,737	12,030	1,630	86.1	12.6	1.32
Low-affinity	1,070	8,120	N.D.	N.D.	N.D.	N.D.
Parent culture	2,112	3,800	539	94.3	4.4	0.25

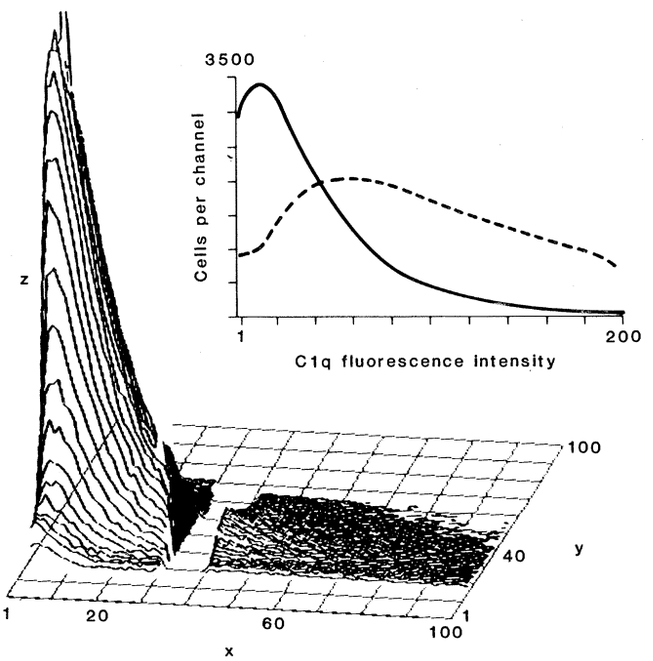

Fig. 1. Confluent monolayers of fibroblasts at passages 4 to 6 maintained in Dulbecco-Vogt medium with 10 percent pooled fresh human serum were made quiescent and synchronous by incubation in a serum-free RPMI 1640 medium (Grand Island Biological) for 48 hours (*3*). The cells were harvested and suspended at 5×10^6 per milliliter in binding buffer (RPMI 1640 and 20 m*M* Hepes, *p*H 7.2, with 1 percent bovine serum albumin plus sufficient 5.75 percent sucrose and 20 m*M* Hepes, *p*H 7.2, to achieve an ionic strength $\dot{U} = 0.07$); the binding sites for C1q were saturated as described (*9*). The cells were then washed, exposed to fluorescein-labeled monospecific antibodies to C1q (*9*), washed again, and suspended in binding buffer containing 10 m*M* EDTA for sorting with a Cytofluorograph 50H/2150 (Ortho Diagnostic Systems). Fluorescence intensity in arbitrary units is shown on the *x*-axis, cell size on the *y*-axis, and relative cell number on the *z*-axis. The cells were sorted into a high–C1q affinity fraction containing the 20 percent most fluorescent cells and a low–C1q affinity fraction containing the remaining cells. Inset: High-affinity cells six transfers after the original sorting (------) were again saturated with C1q, tagged with fluorescent antibodies to C1q, and examined. The control consisted of the parent culture (——). Cell number is plotted against intensity of fluorescence.

Strains of fibroblasts were established from normal human gingiva, and their growth and synthesis activities were characterized by standard procedures (*6, 10*). The cells were made quiescent and synchronous by serum deprivation and suspended in medium with sufficient purified C1q to saturate the receptor sites (*9*). The suspended cells were exposed to fluorescein-labeled monospecific antibodies to C1q (*9*), washed, and separated on the basis of fluorescence intensity into two fractions. One fraction contained the 20 percent most fluorescent cells (designated as the high-affinity fraction) and the other contained the rest of the cells (designated as the low-affinity fraction) (Fig. 1). After 8 to 12 population doublings in vitro, the parent populations and both fractions were again examined in the cell sorter. The mean value for fluorescence intensity (reported as mean channel number) was 67.9 for the parent cultures, 62.1 for the low-affinity cultures (data not shown), and 116.9 for the high-affinity cultures (Fig. 1, inset). Thus the high-affinity cells remained highly fluorescent throughout several cell replications.

The number and affinity of binding sites was measured by Scatchard plot analysis of C1q binding data (*9*). Although the high-affinity and low-affinity cells and the parent cultures manifested

comparable numbers of binding sites (ranging from 1.1×10^6 to 3.8×10^6), the binding affinities (K_a) differed by an order of magnitude, with a value of $2 \times 10^7 M^{-1}$ for the low-affinity cells and the parent cultures and $1.7 \times 10^8 M^{-1}$ for the high-affinity cells.

To assess cell functions, we measured the synthesis of DNA, total protein and collagen, and the production of various collagen types, and we determined the rate of cell growth. The experiments were repeated several times, and representative results are presented as the mean of triplicate cultures (Table 1). The high-affinity cells synthesized about four times as much DNA during a 4-hour treatment with [^{3}H]thymidine as did the parent cultures and about seven times as much as the low-affinity cells (Table 1). Total protein production, assessed after a 20-minute treatment with [^{35}S]methionine, was significantly greater in the high-affinity cells than in the parent cultures and three to four times greater in the high-affinity cells than in the low-affinity cells (Table 1). The proportion of synthesis activity committed to collagen production in the high-affinity cells was about three times that in the parent cultures, with collagen accounting for 40.8 percent and 27.6 percent of total protein in the high-affinity and parent cultures, respectively. The proportion of collagen type I produced by cultures of high-affinity cells and parent cultures was within the range usually seen (*10, 11*) but the production of collagen types III and V was, respectively, about three times and five times greater in the cultures of the high-affinity cells than in the parent cultures (Table 1). In some experiments, production of collagen type V was up to 14 times that in parent cultures.

We have isolated a subset of diploid fibroblasts with unusual phenotypic features. In comparison with the parent cultures from which they were derived, these cells grow and produce proteins at high rates; they commit almost twice as much protein-synthesizing capacity to collagen synthesis, and they produce collagen types III and V in much greater amounts. They possess binding sites for C1q (and therefore presumably for C1) with a significantly higher affinity than that of the parent cultures. All of these features appear to be stable in cultures through at least 12 doublings in our experiment.

Rapid growth and synthesis and the production of large quantities of collagen, especially types III and V, are properties expected of cells in wounds and sites of inflammation. We suggest that factors present at such sites may mitogenically activate one or more subtypes of fibroblast and lead to subtype amplification. Our experiments indicate that C1 may be a key factor in this process and that amplification of a subset of the type we have isolated can occur. As wounds heal and inflammation subsides, and the concentration of C1 decreases, mixtures of subtypes may revert to those characteristic of normal connective tissues.

SANDRA BORDIN
R. C. PAGE
A. S. NARAYANAN
Departments of Pathology and *Periodontics, Schools of Medicine* and *Dentistry,* and *Center for Research in Oral Biology, University of Washington, Seattle 98195*

References and Notes

1. R. W. Holly, *Nature (London)* **258**, 487 (1975); A. B. Pardee, R. Dubrow, J. L. Hamlin, R. F. Kletzien, *Annu. Rev. Biochem.* **47**, 715 (1978); G. Carpenter, *ibid.* **48**, 193 (1979); R. Ross and A. Vogel, *Cell* **14**, 203 (1978); R. F. Diegelman, I. Cohen, A. M. Kaplan, *Proc. Soc. Exp. Biol. Med.* **169**, 445 (1982); R. L. Johnson, M. Ziff, *J. Clin. Invest.* **58**, 240 (1976); T. I. Korotzer, R. C. Page, G. A. Granger, P. S. Rabinovitch, *J. Cell. Physiol.* **111**, 247 (1982).
2. T. I. Korotzer, J. A. Clagett, W. P. Kolb, R. C. Page., *J. Cell. Physiol.* **105**, 503 (1980).
3. S. D. Ko, R. C. Page, A. S. Narayanan, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 3429 (1977).
4. M. D. Hollenberg and P. Cuatrecasas, *ibid.* **70**, 2964 (1973); P. Gordon, J.-L. Carpenter, S. Cohen, L. Orci, *ibid.* **75**, 5025 (1978); K. Glenn, D. F. Bowen-Pope, R. Ross, *J. Biol. Chem.* **257**, 5172 (1982).
5. C. W. Leroy, *J. Exp. Med.* **135**, 1351 (1972); R. F. Diegelmann, I. K. Cohen, B. J. McCoy, *J. Cell. Physiol.* **98**, 341 (1979); K. Peitile and T. Nikkari, *Atherosclerosis* **37**, 11 (1980).
6. T. M. Hassell, R. C. Page, A. S. Narayanan, G. C. Cooper, *Proc. Natl. Acad. Sci. U.S.A.* **73**, 2909 (1976); A. S. Narayanan and R. C. Page, *Lab. Invest.* **39**, 61 (1978).
7. A. S. Narayanan and R. C. Page, *Collagen Rel. Res.* **3**, 33 (1983).
8. G. M. Martin, C. A. Sprague, T. H. Norwood, W. R. Pendergrass, *Am. J. Pathol.* **74**, 137 (1974); J. R. Smith and R. G. Whitney, *Science* **207**, 82 (1980); C. E. Brinckeroff and Y. E. Nagels, *Collagen Rel. Res.* **1**, 433 (1981); G. R. Botstein, G. K. Sherer, E. C. LeRoy, *Arthritis Rheum.* **29**, 189 (1982); Y. H. Korn, *J. Clin. Invest.* **71**, 1240 (1983).
9. S. Bordin, W. P. Kolb, R. C. Page, *J. Immunol.* **130**, 1871 (1982).
10. A. S. Narayanan and R. C. Page, *J. Biol. Chem.* **251**, 5414 (1976).
11. J. Huey, A. S. Narayanan, K. Jones, R. C. Page, *Biochim. Biophys. Acta* **632**, 227 (1980).
12. A. S. Narayanan and R. C. Page, *J. Biol. Chem.* **258**, 11694 (1983).
13. Supported by NIH grants DE-03301 and DE-02600. The assistance of P. S. Rabinovitch with the fluorescence-activated cell sorter is gratefully acknowledged.

2 September 1983; accepted 25 October 1983

Mapping of the Human *Blym*-1 Transforming Gene Activated in Burkitt Lymphomas to Chromosome 1

Abstract. Blym-*1, a transforming gene detected by transfection of NIH 3T3 cells with DNA from Burkitt lymphomas, was mapped to the short arm of chromosome 1 (1p32) by chromosomal in situ hybridization. The* Blym-*1 gene was not physically linked to the cellular* myc *oncogene or to any of the immunoglobulin gene loci implicated in the characteristic chromosomal translocations in Burkitt lymphoma.*

Molecular genetic analyses of several human Burkitt lymphoma cell lines have revealed DNA rearrangements in which there has been a translocation between the cellular oncogene c-*myc* and immunoglobulin gene loci (*1*). The characteristic chromosomal translocations observed in Burkitt lymphomas [t(2;8)(p12;q24), t(8;14)(q24;q32), and t(8;22)(q24;q11)] (*2*) indicated the potential importance of a gene locus on the long arm of chromosome 8 at band q24 (8q24), the band to which the c-*myc* gene was subsequently mapped (*1, 3*). Furthermore, several investigators suggested that the significance of the specific translocations to malignant transformation may have resulted from the alteration of cellular transforming genes by their juxtaposition with specific DNA sequences, that is, immunoglobulin gene sequences (*4*).

Recently a transforming gene has been detected by transfection of NIH 3T3 cells with DNA from six Burkitt lymphomas (*5*). This gene is homologous by molecular hybridization with the *Blym*-1 transforming gene isolated from chicken B-cell lymphoma DNA (*6*), but is not homologous with 12 retroviral transforming genes including v-*myc* and v-*ras*. The involvement of these two distinct genes in B-cell lymphomas of both chickens and humans further supports the hypothesis that c-*myc* and *Blym*-1 may be involved in different stages of progression to neoplasia (*7*).

We were interested in determining the chromosomal location of the human *Blym*-1 transforming gene and its possible relation to c-*myc* or the immunoglobulin gene loci, loci known to be active in cells from Burkitt lymphomas. By using chromosomal in situ hybridization we were able to assign the human *Blym*-1 gene to the short arm of chromosome 1 (1p32) (Fig. 1), a locus unlinked to either c-*myc* or immunoglobulin gene sequences.

The probe we used for the chromosome hybridization was a plasmid containing the ^{3}H-labeled 0.95-kb biologically active Eco RI fragment of the human