phasize the relation to the Gram-positive bacteria. For example, of the eight possible specific sequences of the general form UAACCYYY . . . , five are seen among the catalogs of the "clostridial" subdivision of the Gram-positive bacteria, and these represent at least six phylogenetically independent occurrences. Similarly, six of the eight possible versions of YAAYACCCR are found in this same group, representing at least eight phylogenetically independent occurrences (*10*).

Heliobacterium chlorum appears closer to the "clostridial" (low G + C) than to the "actinomycete" (high G + C) subdivision (*3*) of the Gram-positive bacteria by this measure. Although the organism should probably be considered a member of this particular subdivision, a definitive statement regarding this must await full sequence analysis of a representative "actinomycete," because the relative lack of closeness in this case may merely reflect rapid evolution in the "actinomycete" subline (*11*). In Table 1, *H. chlorum* also shows some relationship to the spirochete "phylum" (*12*). A possible distant but specific relationship between the spirochetes and Gram-positive bacteria has previously been noted (*4*).

To date nothing we know about the *H. chlorum* phenotype suggests a relationship to the Gram-positive bacteria (or to any other eubacteria for that matter). On preliminary examination, the organism's cell wall is not of the Gram-positive type (*13*).

The association of *H. chlorum* with the Gram-positive bacteria is surprising only in terms of our prejudices regarding relationships between photosynthetic and nonphotosynthetic bacteria. Precedent for the observed relationship definitely exists in the phylogeny of eubacteria determined by rRNA cataloging (*3–4*). The purple photosynthetic bacteria are part of a unit, a phylum, that includes many nonphotosynthetic phenotypes (*5*). The known members of the green nonsulfur "phylum" are predominantly nonphotosynthetic (*6*). The interesting question now is whether there exist other, as yet undiscovered, photosynthetic eubacteria belonging to "phyla" not now known to contain photosynthetic species.

Given that five of the ten recognized eubacterial "phyla" (*4*) have now been shown to contain (each a different type of) photosynthetic species, it would seem likely that the ancestor common to all eubacteria was itself photosynthetic. The point, although strengthened by our results, is, however, intrinsically unprovable. Therefore, although our conclusion is not a compelling one, it does demand that the archaic and now suspect notion that all bacteria have arisen from a common nonphotosynthetic ancestor no longer be accepted and perpetuated as dogma.

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25. Supported by grants from NASA and NSF (C.R.W.) and by the Gesellschaft fuer Biotechnologische Forschung to support the German Collection of Microorganisms (E.S.).

5 March 1985; accepted 30 April 1985

Antibody-Directed Urokinase: A Specific Fibrinolytic Agent

Abstract. *A specific fibrinolytic agent was synthesized by covalently coupling urokinase to a monoclonal antibody that was fibrin-specific and did not cross-react with fibrinogen. The antibody was raised against a synthetic peptide representing the seven amino-terminal residues of the beta chain of human fibrin. The urokinase-antifibrin conjugate retained the original binding specificity of the antibody and showed 100-fold increased fibrinolysis in vitro when compared to unmodified urokinase. The presence of human fibrinogen at plasma concentration did not influence these properties.*

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Coronary arteriographic studies indicate that 87 percent of transmural myocardial infarctions are caused by coronary thrombosis (*1*). Although thrombolytic agents currently available can lyse coronary artery thrombi in the early hours of coronary thrombosis and thereby diminish myocardial injury, their clinical application has been attended by significant problems. Both urokinase and streptokinase activate the conversion of plasminogen to the fibrinolytic enzyme plasmin. Plasmin, in turn, not only affects lysis of the fibrin in the thrombus but also promotes generalized fibrinogenolysis, at times resulting in severe bleeding (*2*). Human tissue plasminogen activator may be more fibrin-specific than urokinase (*3*). In order to target urokinase to a fibrin-containing clot, we coupled this plasminogen activator to a monoclonal antibody that was raised against a synthetic peptide representing the seven amino-terminal residues of the beta chain of human fibrin. This antibody is specific for fibrin and does not cross-react with fibrinogen (*4*). Fibrinolysis was promoted to a much greater extent with the conjugate than with unmodified urokinase.

Reduced urokinase was coupled to fibrin-specific monoclonal antibody 64C5 by means of its intrinsic sulfhydryl groups, with *N*-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) as a cross-linking agent (*5*). The cross-linking agent (20 m*M* in 0.05 ml of absolute ethanol) was added to the antibody [6.3 mg in 3.0 ml of phosphate-buffered saline (PBS)

consisting of 0.1*M* sodium phosphate and 0.1*M* NaCl, *p*H 7.4], and the mixture was allowed to react for 30 minutes at room temperature. The solution was subsequently dialyzed three times against 1 liter of PBS.

Analysis for 2-pyridyldisulfide content (*6, 7*) showed 10.8 residues per antibody molecule. Urokinase (7 mg; 3.5 mg per milliliter in 0.1*M* sodium acetate and 0.1*M* NaCl, *p*H 4.5) was combined with 20 μCi of ^{125}I-labeled urokinase [0.03 mg in 0.3 ml of PBS containing 0.2 percent NaN_3, phosphate-buffered saline azide (PBSA)] (*8*). The mixture was reduced by addition of 0.23 ml of 1.0*M* dithiothreitol in 0.1*M* sodium acetate and 0.1*M* NaCl (*p*H 4.5) for 30 minutes at room temperature and desalted on Sephadex G-25 (0.7 by 25 cm) equilibrated with PBSA (*p*H 4.5). Peak fractions from the column were pooled (4.3 ml; 1.1 mg per milliliter of protein) (*9*) and mixed with the 3-(2-pyridyl)propionyl derivative of the antibody (PDP-antibody, 2.9 ml containing 2.1 mg per milliliter of protein) (*9*). The mixture was neutralized and allowed to react overnight. Under these conditions the intrinsic sulfhydryl groups of the urokinase chains react with the pyridyldisulfide groups of the modified antibody, resulting in displacement of thiopyridine and formation of a disulfide-containing intermolecular bridge.

Unconjugated urokinase and its component subunits were separated from the ^{125}I-labeled urokinase-antibody conjugate (urokinase-antifibrin conjugate) by gel filtration on Sephacryl S-200 (2.5 by 90 cm, with PBSA as elution buffer). Two radioactive fractions were clearly resolved, the first of which contained the antibody-urokinase conjugate and was free of unconjugated urokinase. The molecular size of this fraction was greater than 150 kilodaltons, as assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis, and it proved to be radioactive on subsequent autoradiography. Since radioactivity was initially associated with the 30-kD subunit of urokinase, this observation demonstrated the covalent linkage of urokinase to antibody. The incorporation of urokinase averaged 1 mol per 3 mol of antibody, as determined by the specific radioactivity of the urokinase subunit. Further evidence of the association of urokinase activity with antibody was obtained by affinity chromatography of the antibody-urokinase conjugate on a column constructed by coupling a synthetic amino-terminal β-chain fibrin peptide (Gly-His-Arg-Pro-Leu-Asp-Lys-Cys) (β-peptide) (*4*) to maleimidobenzoyl lysine–Sepharose Cl-4B (*10*). The eluate of this column (0.2*M* glycine HCl, *p*H 2.8) was radioactive and fibrinolytic, both properties of urokinase, in the assay described below. The same methods were used to synthesize and purify a conjugate of urokinase and another monoclonal antibody of the same isotype (control conjugate) (*11*).

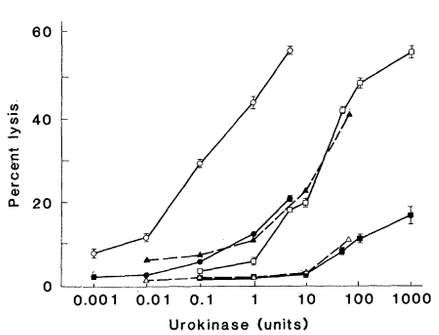

Fig. 1. Release of labeled peptides from fibrin-Sepharose by a conjugate of urokinase and fibrin-specific antibody (urokinase-antifibrin conjugate) (●, 2.5 hours; ○, 15 hours), urokinase control conjugate (△, 2.5 hours; ▲, 15 hours), and unconjugated urokinase (■, 2.5 hours; □, 15 hours). The two conjugates and the unconjugated urokinase (100 μl containing the indicated amount of urokinase) were each incubated for 4 hours with 100 μl of ^{125}I-labeled fibrin–Sepharose; washed once with 3 ml of 0.1*M* tris, 0.1*M* NaCl, 0.5 percent bovine serum albumin, and 0.5 percent Triton X-100 and three times with 3 ml of TBSA; and incubated for 2.5 and 15 hours with purified plasminogen (120 mg/liter). Lysis was expressed as the quotient of released radioactivity and total radioactivity. Each point represents the mean ± standard deviation of three separate experiments.

A quantitative fibrinolytic assay was devised by linking fibrin monomer to Sepharose. Human fibrinogen (Kabi grade L, 500 mg) was dissolved in 50 m*M* phosphate buffer (*p*H 7.4) and then passed over lysine-Sepharose to eliminate traces of plasminogen. The resulting fibrinogen was mixed with 150 μCi of ^{125}I-labeled fibrinogen (IBRIN) and coupled to 150 ml of cyanogen bromide–activated Sepharose Cl-4B. After being thoroughly washed, the gel was suspended in 0.1*M* tris, 0.15*M* NaCl, and 0.02 percent NaN_3 (*p*H 7.4) (TBSA), and the immobilized fibrinogen was converted to fibrin by addition of human thrombin (1 NIH unit per milliliter) in the presence of 100 m*M* $CaCl_2$. After being washed with 4 liters of TBSA, ^{125}I-labeled fibrin–Sepharose was stored in TBSA at 4°C. The substituted Sepharose was judged to be stable on incubation with plasminogen in the absence of urokinase or conjugates containing urokinase, releasing 0.1 percent of its radioactivity at 2.5 hours and 2.1 percent at 15 hours.

To assess their relative fibrinolytic activity, we incubated increasing amounts of urokinase-antifibrin conjugate and unconjugated urokinase with 100 μl of ^{125}I-fibrin–Sepharose for 4 hours. The Sepharose was washed first with 3 ml of a solution consisting of 0.1*M* tris, 0.1*M* NaCl, 0.5 percent bovine serum albumin, and 0.5 percent Triton X-100, then three times with 3-ml aliquots of TBSA. Thereafter the resin was incubated at room temperature with purified plasminogen (*12*) (0.12 mg/ml) in 50 m*M* phosphate buffer (*p*H 7.4). After intervals of 2.5 and 15 hours, the mixture was centrifuged, and the radioactivity of the supernatant was determined in a gamma scintillation counter. This procedure was repeated with the control conjugate.

Kinetic information was obtained as follows: control conjugate and urokinase-antifibrin conjugate, each in TBSA containing plasminogen (0.12 mg/ml), were continuously circulated at a rate of 1 ml per minute over a column (0.3 by 5 cm) containing 300 μl of ^{125}I-labeled fibrin–Sepharose. At set intervals, three samples of 1 ml each were collected and their radioactivity was determined.

Figure 1 indicates that the concentration of urokinase-antifibrin conjugate required to release labeled peptides from

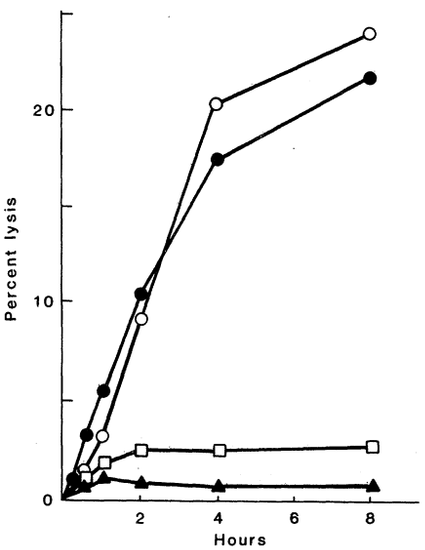

Fig. 2. Release of ^{125}I-labeled peptides from fibrin-Sepharose during continuous circulation of a solution containing plasminogen (0.12 mg/ml) and urokinase-antifibrin conjugate in the presence (○) and absence (●) of fibrinogen (3.5 mg/ml). The experiment was repeated with control conjugate in the presence (▲) and absence (□) of fibrinogen. In each instance the circulating fluid contained urokinase (0.25 units per milliliter) coupled to either of the antibodies. Each point represents the mean of three determinations of a single experiment with a standard deviation of less than 1.6 percent.

fibrin-Sepharose is 1/100 of that of unconjugated urokinase, at both 2.5 and 15 hours. The control conjugate does not differ significantly from unconjugated urokinase. These results have been reproduced in separate experiments with three different conjugate preparations. Urokinase-antifibrin conjugate enhances the rate of release of peptides from fibrin-Sepharose, and this effect is unimpaired by fibrinogen at a physiologic concentration (Fig. 2). β-Peptide inhibits fibrinolysis of urokinase-antifibrin conjugate, whereas it has no effect on the fibrinolytic rate of unconjugated urokinase or control conjugate. In the presence of β-peptide (1.5 mg/ml) during the incubation of urokinase with fibrin-Sepharose, 82 percent of the increase in fibrinolysis is blocked. Taken together, the data in Fig. 2 and the β-peptide inhibition of increased fibrinolysis are consistent with the specificity of antibody 64C5 (*4*).

Thus a monoclonal antibody specific for fibrin is able to target the plasminogen activator urokinase to fibrin and, by virtue of enhanced local concentration, increase the efficiency of plasmin lysis by a factor of 100. The antibody is sufficiently fibrin-specific that physiologic concentrations of fibrinogen do not interfere with enhanced fibrinolysis. Fibrinolytic effectiveness is not increased by the coupling of urokinase to a monoclonal antibody of irrelevant specificity, and it is diminished by a peptide representing the epitope recognized by the fibrin-specific antibody. If these effects can be demonstrated in vivo, the dose of urokinase required for effective lysis of a thrombus would result in only minimal fibrinogenolysis, the major impediment to the widespread application of this form of therapy.

Note added in proof: Preliminary reports of a similar nature have been reported (*13*).

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14. We thank J. Devine, M. Terwilliger, and J. Watabe for technical assistance and T. McVarish for editorial assistance. Supported by a grant from the Schering Corporation. C.B. was supported by a fellowship from Boehringer-Ingelheim.

19 March 1985; accepted 2 July 1985

The Role of the c-*mos* Gene in the 8;21 Translocation in Human Acute Myeloblastic Leukemia

Abstract. *The human* c-mos *proto-oncogene is located on chromosome 8 at band q22, close to the breakpoint in the t(8;21) (q22;q22) chromosome rearrangement. This translocation is associated with acute myeloblastic leukemia, subgroup M2. The* c-myc *gene, another proto-oncogene, has been mapped to 8q24. The breakpoint at 8q22 separates these genes, as determined by in situ hybridization of* c-mos *and* c-myc *probes. The* c-mos *gene remains on the 8q− chromosome and the* c-myc *gene is translocated to the 21q+ chromosome. Southern blot analysis of DNA from bone marrow cells of four patients with this translocation showed no rearrangement of* c-mos.

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The cellular homolog (c-*mos*) of the transforming sequence (v-*mos*) of the Moloney murine sarcoma virus has transforming activity when linked to a viral long terminal repeat (LTR) and transfected into NIH 3T3 cells (*1*). Transcriptional activation of c-*mos* has also been observed in non–virally induced mouse plasmacytomas XRPC-24 (*2*) and MOPC-21 (*3, 4*) after insertion of an LTR (from an intracisternal A-type particle) at the 5′ end of the c-*mos* gene coding region. Apart from these two cell lines, transcription of c-*mos* has not been observed in various normal or malignant cells (*5, 6*) and c-*mos* has not been associated with spontaneously developing malignancies. The human c-*mos* gene has been cloned from human genomic libraries (*7–9*) and assigned to chromosome 8 by Southern blot hybridization to DNA from somatic cell hybrids. By means of in situ hybridization between a human c-*mos* probe and human meiotic chromosomes, the gene was localized to band q22 (*10*); this location is proximal to the locus of the c-*myc* gene, which had previously been assigned to band q24 (*10*). The c-*myc* gene is frequently rearranged and is transcriptionally activated in the t(8;14) (q24;q32) translocation of Burkitt lymphoma after juxtaposition with the immunoglobulin gene sequences (*11*). The chromosomal band to which c-*mos* has been mapped is also the site of one of the breakpoints in the t(8;21)(q22;q22) of acute myeloblastic leukemia (AML) (*12, 13*). We have begun a study of this translocation to explore the possibility that c-*mos* may be activated by the rearrangement.

The 21q+ chromosome (which contains the distal portion of chromosome 8) from malignant cells of a patient having the M2 subtype of AML (AML with maturation) and a t(8;21) carries the translocated c-*myc* gene but lacks the c-*mos* gene (*14*). We now provide evidence from two additional cases that the c-*mos* gene remains on the 8q− derivative chromosome after the t(8;21), whereas the c-*myc* gene is translocated to the 21q+ chromosome. To detect possible rearrangements of c-*mos* due to the translocation breakpoint, we have also analyzed the genomic DNA from the malignant cells of four AML patients with a t(8;21).

DNA probes were derived from (i) a human genomic clone containing c-*mos* coding and flanking sequences within a 2.7-kilobase (kb) Eco RI insert in pBR322 (*8*) and (ii) a complementary DNA clone containing part of the second and all of the third exon of the c-*myc* gene in a 1.03-kb insert cloned in pBR322 (*15*). The ^{3}H-labeled probes were prepared from the complete plasmids by nick-translation and were used for in situ hybridization (Table 1 and Fig. 1). When the two probes were hybridized to normal metaphase cells from peripheral blood lymphocytes, specific labeling of