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Nonlethal Adherence to Human Neutrophils Mediated by Dr Antigen-Specific Adhesins of *Escherichia coli*

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Uropathogenic *Escherichia coli* strains express a variety of adhesins, including members of the Dr adhesin family such as the Dr hemagglutinin, AFAI, and AFAIII. Certain *E. coli* adhesins (e.g., type 1 and S fimbriae) are known to mediate adherence to human polymorphonuclear leukocytes (PMNs). The receptor on erythrocytes for Dr family adhesins, decay accelerating factor, is also present on PMNs. To determine whether Dr family adhesins mediate adherence to PMNs and to characterize the specificity and consequences of such adherence, we studied agglutination of PMNs and adherence to PMNs by recombinant *E. coli* strains expressing various mannose-resistant or mannose-sensitive adhesins, in the presence or absence of inhibitors of adherence. Dr family adhesins, like type 1 fimbriae, mediated concentration-dependent adherence to PMNs. Adherence to PMNs was mannose sensitive for type 1 fimbriae but mannose resistant for Dr family adhesins. Chloramphenicol inhibited PMN adherence for the Dr hemagglutinin with the same potency as that with which it inhibited hemagglutination, but it was inactive against PMN adherence and hemagglutination mediated by other members of the Dr adhesin family. In contrast to PMN adherence mediated by type 1 fimbriae, adherence mediated by the Dr hemagglutinin did not lead to significantly increased bacterial killing. These data suggest that Dr family adhesins mediate a novel pattern of adherence to PMNs, probably by recognizing decay accelerating factor, with minimal consequent bacterial killing.

Uropathogenic *Escherichia coli* strains are characterized by the expression of a distinctive array of adhesins, including type 1, P, S, and G fimbriae (as reviewed in reference 17) and nonfimbrial adhesins such as those of the Dr family (the Dr hemagglutinin, AFAI, and AFAIII) (17) and the NFA (nonfimbrial adhesin) series (12, 14–16, 22). In addition to their roles in facilitating colonization of host mucosal surfaces (17), several of these adhesins also mediate interactions with host phagocytes that may significantly influence the pathogenesis of urinary tract infection (17). Type 1 fimbriae mediate mannose-sensitive adherence to human polymorphonuclear leukocytes (PMNs) (5, 41, 48, 50), which stimulates the respiratory burst and release of granular contents (11, 29, 47) and can result in nonopsonic phagocytosis (lectinophagocytosis) of the bacteria (40, 41, 45, 53). S fimbriae augment the ability of hemolytic *E. coli* strains to stimulate mediator release from PMNs, presumably by mediating bacterial attachment to PMNs (20). The nonfimbrial adhesin NFA-1 also mediates adherence to and stimulation of PMNs (13). In contrast, P fimbriae do not mediate adherence to PMNs (48, 52), probably because PMNs lack membrane glycolipid receptors for P fimbriae (6, 28, 48).

Adhesins of the Dr family are associated with pathogenic *E. coli* causing urinary tract infection (the Dr hemagglutinin, AFAI, and AFAIII) (23–25, 33) and diarrhea (F1845 fimbriae) (4). These adhesins have a common genetic basis (36) and recognize distinct receptor epitopes on decay accelerating factor (DAF) (34, 36, 37), a phosphotidylinositol-linked membrane glycoprotein involved in regulation of the complement cascade that contains the human Dr blood group antigen (2, 7, 27, 49). Strains expressing Dr-specific adhesins agglutinate Dr-positive human erythrocytes but fail to agglutinate Dr-negative erythrocytes that lack DAF (37). The designation Dr adhesins is based on this Dr blood group antigen specificity (36, 37). Chloramphenicol (and structurally related tyrosine analogs) inhibit adherence mediated by the Dr hemagglutinin and by some strains expressing AFAIII (26), but not by other adhesins of the Dr family (36, 37).

Receptors for the Dr hemagglutinin are widely distributed in human digestive, urinary, genital, and respiratory tracts and skin (19, 21, 35, 39). The presence of such receptors on renal tubular basement membranes, Bowman's capsule, and transitional epithelium has been proposed to underlie the importance of Dr adhesin-positive *E. coli* in ascending colonization of the urinary tract (21, 34, 39). The Dr adhesin receptor DAF is also present on the surfaces of PMNs (31, 32). However, it is not known whether receptor epitopes for Dr family adhesins are conserved on the molecular form of DAF present on PMNs and whether the number, distribution, and accessibility of DAF molecules on PMNs is sufficient to support adherence by Dr adhesin-positive bacteria.

Because of the potential pathogenic importance of pathogen-phagocyte interactions (40), we undertook this study to evaluate the hypothesis that *E. coli* adhesins of the Dr family mediate adherence to PMNs and to compare the specificity of such adherence with the DAF-specific adherence to erythrocytes that these adhesins mediate. We also sought to determine whether adherence to PMNs by Dr adhesin-positive *E. coli* strains promotes phagocytosis and bacterial killing in the same fashion as with type 1 fimbriae, thus potentially constituting a liability for the pathogen and providing a survival advantage to adhesin-negative phase variants.

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TABLE 1. Bacterial strains used in this study

Strain	Recombinant plasmid content	Host strain	Adhesin(s)[a]		MR adhesin phenotype[b]	Selection[c]	Reference or source
			MS	MR			
IH11128	None[d]	NA[d]	+/−	+	Dr	None	33
BN406	pBJN406	EC901	+	+	Dr	Chl^r	33
EC901	None[e]	NA[e]	+	−	NA	None	33
HB101/pILL22	pILL22	HB101	+/−	+	AFAI	Amp^r	25
HB101/pILL115	pILL115	HB101	+/−	+	AFAIII	Amp^r	36
HB101	None[e]	NA[e]	+/−	−	NA	None	4
P678-54/pSSS1	pSSS1	P678-54	−	+	F1845	Amp^r	This study
P678-54/pJJ48	pJJ48	P678-54	−	+	P	Amp^r	This study
SH48	pSH2	P678-54	+++	−	NA	Chl^r	42
P678-54	None[e]	NA[e]	−	−	NA	None	42

[a] MS, mannose-sensitive agglutination of *S. cerevisiae*; MR, mannose-resistant agglutination of human P_1 erythrocytes. For MS adhesins: +++, very strong; ++, strong; +, moderate; +/−, weak; −, absent agglutination. For MR adhesin: + and −, absolute presence and absence, respectively, of MR phenotype only.
[b] Dr, the Dr hemagglutinin; AFA, afimbrial adhesin; P, P fimbriae; F1845, F1845 fimbriae; NA, not applicable (no MR adhesin).
[c] The resistance phenotype which was selected for is given. Chl, chloramphenicol; Amp, ampicillin.
[d] Strain is wild-type isolate; NA, not applicable.
[e] Strain is laboratory host strain.

MATERIALS AND METHODS

Plasmids and strains used. Wild-type and recombinant strains expressing type 1 fimbriae, P fimbriae, and adhesins of the Dr family (the Dr hemagglutinin, AFAI, AFAIII, and F1845 fimbriae) were studied (Table 1). For recombinant strains expressing cloned adhesin determinants, the corresponding adhesin-negative host strains were included (Table 1). Strains P678-54/pJJ48 and P678-54/pSSS1 were created by transforming plasmids pJJ48 (18) and pSSS1 (4) into host strain P678-54 (42) by standard methods (30). Additional recombinant strains were created by transforming the other MR adhesin-encoding plasmids shown in Table 1 into the adhesin-negative host strain P678-54; however, the MR phenotype of these transformants was too weak and variable for use in adherence and adherence inhibition studies (data not shown). Strains were grown on Luria-Bertani (LB) agar (30) supplemented with appropriate antibiotics as needed for recombinant strains (Table 1).

Neutrophil isolation. For agglutination and adherence assays, PMNs were obtained from EDTA- or heparin (2 U/ml)-anticoagulated venous blood of normal healthy adult volunteers and of a patient with the severe phenotype of leukocyte adhesion deficiency (congenital absence of CD18 and thus of the putative receptor on PMNs for type 1 fimbriae, CR3 [CD116 and CD18] [40]). PMNs were isolated by dextran sedimentation, hypotonic lysis of residual erythrocytes, and Ficoll-Hypaque density-gradient centrifugation, as previously described (44, 46). Cells were suspended in Hanks' balanced salt solution without calcium or magnesium (HBSS) at approximately 2×10^7 PMNs per ml. Cell preparations were ≥95% neutrophils by Diff-Quick staining, and viability was ≥96% by trypan blue exclusion.

Inhibitors. Solutions of inhibitor substances (from Sigma Chemical Corp., St. Louis, Mo.) were prepared in phosphate-buffered saline (PBS; pH 7.4) and included D-mannose (1.1 M), methyl-α-D-mannoside (1.1 M), and *p*-nitrophenyl α-D-mannoside (2.5 mM) as inhibitors of type 1 fimbrial adherence (10) and chloramphenicol (5 mM) and *t*-butoxycarbonyl-*O*-benzyl-L-tyrosine (BCBT; 2.5 mM) as inhibitors of the Dr hemagglutinin (39). Whole pigeon egg white and globoside (2.5 mM) in phosphate-buffered saline (PBS) were prepared as previously described (18) as inhibitors of P-fimbrial adherence.

HA, NA, and agglutination inhibition assays. Slide hemagglutination (HA) assays were done by use of suspensions of plate-grown bacteria and human P_1 erythrocytes, with or without inhibitor solutions, as previously described (18). Neutrophil agglutination (NA) assays were done in the same manner as HA assays, but with the use of PMNs rather than erythrocytes and with bacteria-free (i.e., PMN-only) controls interspersed between reaction mixtures containing bacteria to control for spontaneous clumping of PMNs. Assays were done at room temperature in all cases and also on ice when agglutination reactions were weak. The minimal agglutinating concentration (MAC; lowest bacterial concentration giving macroscopic agglutination) for HA and NA was determined as previously described for HA (18). Agglutination inhibition was tested at four times the MAC, by use of serial twofold dilutions of the inhibitor solutions in PBS. For inhibitors giving complete inhibition of HA or NA, the 50% inhibitory concentration (IC_{50}) was determined as previously described (18).

PMN adherence assay. Adherence of *E. coli* to PMNs was assessed by direct visualization with fluorescence microscopy, as described elsewhere (3, 8). Bacteria were grown overnight on LB agar with antibiotics added as needed, scraped from a plate, and suspended in 250 μl of HBSS. Bacteria were diluted 1:10 in 50 mM sodium carbonate buffer, pH 9.6, and labeled with 0.1% fluorescein isothiocyanate (Sigma) for 30 min at 37°C while protected from light. Labeled *E. coli* were washed twice and suspended in HBSS.

Each adherence mixture contained 50 μl of fluorescein-labeled *E. coli* cells (1×10^{10} to 5×10^{10}/ml, unless otherwise noted) and 50 μl of PMNs (2×10^7/ml) in HBSS plus 1 mM Ca^{2+} and 1 mM Mg^{2+}. For inhibition experiments, inhibitors (final concentration, 5 mM chloramphenicol or 100 mM methyl-α-D-mannoside, unless otherwise noted) were added to labeled bacteria prior to the addition of PMNs. *E. coli*-PMN mixtures were tumbled at 8 rpm in the dark at 4°C for 90 min. Tubes were momentarily spun in a tabletop microfuge to sediment PMNs, the supernatant was removed, and PMNs were resuspended in 100 μl of HBSS. Five milliliters of 0.1% ethidium bromide was added to a final concentration of 50 μg/ml, and 10 μl of the mixture was placed on a glass slide, overlaid with a coverslip, and examined within 20 min with a Nikon Optiphot fluorescence microscope with a 520-nm fluorescein isothiocyanate filter under oil immersion (magnification, × 1,000). Surface-attached or unattached extracellular *E. coli* appeared orange or green with orange centers, whereas ingested organisms showed a rim of intense green fluorescence without any orange staining. Twenty-five consecutive individual PMNs per sample were examined, and the number of adherent bacteria was determined as the number of attached or ingested organisms per cell. Each strain was tested with PMNs from at least two different donors, and the results were averaged. We have verified the accuracy of the fluorescence microscopy method in distinguishing intracellular from extracellular organisms for enterococci by transmission electron microscopy (3).

PMN microbicidal system. *E. coli* (approximately 5×10^7 CFU/ml) was incubated with PMNs (1×10^7/ml) in HBSS plus 1 mM Ca^{2+} and 1 mM Mg^{2+} in final volumes of 200 μl in microtiter plate wells and agitated at 37°C. At intervals, 20-μl aliquots were diluted 1:10 in water for 10 min to lyse PMNs and then serially diluted, and viability was determined by the pour plate method with LB agar. To compare the effects on bacterial killing by PMNs of serum opsonization and of adhesin expression, in some experiments 10% serum from a patient with C8 deficiency was added to *E. coli*-PMN mixtures to provide opsonization without complement-mediated lysis. (Preliminary experiments showed that in the absence of PMNs these strains of *E. coli* were killed by normal serum alone but were resistant to killing by C8-deficient serum; data not shown).

Statistical methods. Statistical significance was assessed with the use of Student's two-tailed *t* test.

RESULTS

HA, NA, and inhibition of HA and NA. As an initial approach to evaluating the binding of Dr family adhesins to PMNs, agglutination reactions with PMNs and strains expressing representatives of the Dr adhesin family and other well-studied *E. coli* adhesins were done. HA assays, a well-established method for assessing adhesin phenotypes among uropathogenic *E. coli* strains, were done in parallel to permit comparisons between erythrocytes and PMNs as targets for adherence by the various adhesins. Inhibitors with known specificities for the various adhesins were used to probe the specificities of adherence mediated by Dr family adhesins.

Strains exhibited patterns of HA and HA inhibition consistent with the known specificities of their respective adhesins and with the recognized activities of the various inhibitors used (Table 2). NA and NA inhibition results were similar to the

TABLE 2. Qualitative agglutination and agglutination inhibition

Type of agglutination and adhesin(s)	Agglutination in presence of:			
	PBS	D-Mannose[a]	Pigeon egg white[b]	Chloramphenicol[c]
HA				
Type 1 fimbriae[d]	+	−	+	+
P fimbriae	+	+	−	+
Dr hemagglutinin[e]	+	+	+	−
AFAI, AFAIII, F1845[f]	+	+	+	+
None[g]	−	−	−	−
NA				
Type 1 fimbriae[d]	+	−	+	+
P fimbriae	−	−	−	−
Dr hemagglutinin[e]	+	+	+	−
AFAI, AFAIII, F1845[f]	+	+	+	+
None[g]	−	−	−	−

[a] Methyl-α-D-mannoside and *p*-nitrophenyl-α-D-mannoside gave results similar to those of D-mannose.
[b] Globoside gave results similar to those of pigeon egg white.
[c] BCBT gave results similar to those chloramphenicol.
[d] Strain SH48 (P678-54/pSH2).
[e] Strains IH11128 and BN406 gave similar results.
[f] Strains expressing AFAI (HB101/pI1122), AFAIII (HB101/pILL115), and F1845 (P678-54/pSSS1) gave similar results.
[g] Host strains P678-54, EC901, and HB101 (considered here as adhesin negative) mediated neither hemagglutination nor neutrophil agglutination.

corresponding HA and HA inhibition results for strains expressing type 1 fimbriae or adhesins of the Dr family (Fig. 1; Table 2). In contrast, whereas the P-fimbriated strain mediated HA in a mannose- and chloramphenicol-resistant but pigeon egg white- and globoside-sensitive fashion, it did not agglutinate PMNs even in the absence of inhibitors (Table 2). The laboratory host strains agglutinated neither erythrocytes nor neutrophils (Table 2), despite weak or moderate agglutination of yeast cells by strains HB101 and EC901, respectively (Table 1).

Of note, only the type 1 fimbrial inhibitors D-mannose, methyl-α-D-mannoside, and *p*-nitrophenyl-D-mannoside inhibited HA and NA due to type 1 fimbriae. In contrast, these inhibitors had no effect on HA or NA due to other adhesins (Table 2). Only the P-fimbrial inhibitors pigeon egg white and globoside blocked P-fimbrial HA; these substances had no effect on HA or NA due to other adhesins. The Dr hemagglutinin-specific inhibitors chloramphenicol and BCBT (but none of the other inhibitors) blocked both HA and NA mediated by the Dr hemagglutinin (Fig. 1; Table 2). In contrast, these inhibitors were without effect on HA or NA due to other adhesins, including other members of the Dr adhesin family (Table 2).

HA inhibition and NA inhibition were compared quantitatively by determination of the IC_{50}s of each of the inhibitors for HA and NA, by use of both a Dr hemagglutinin-expressing strain and, as a control, a type 1-fimbriated strain (Table 3). With the type 1-fimbriated strain, D-mannose and methyl-α-D-mannoside had nearly identical IC_{50}s for NA and HA, whereas *p*-nitrophenyl-D-mannoside's IC_{50} was 10-fold higher for NA than for HA (Table 3). As noted above, in qualitative agglutination inhibition assays (Table 2), other inhibitors were ineffective against both HA and NA mediated by type 1 fimbriae, even at maximal concentrations (Table 3). Similarly, with a Dr hemagglutinin-expressing strain, the active inhibitors chloramphenicol and BCBT had nearly identical IC_{50}s for NA and HA; in contrast, other inhibitors were ineffective against both NA and HA (Table 3).

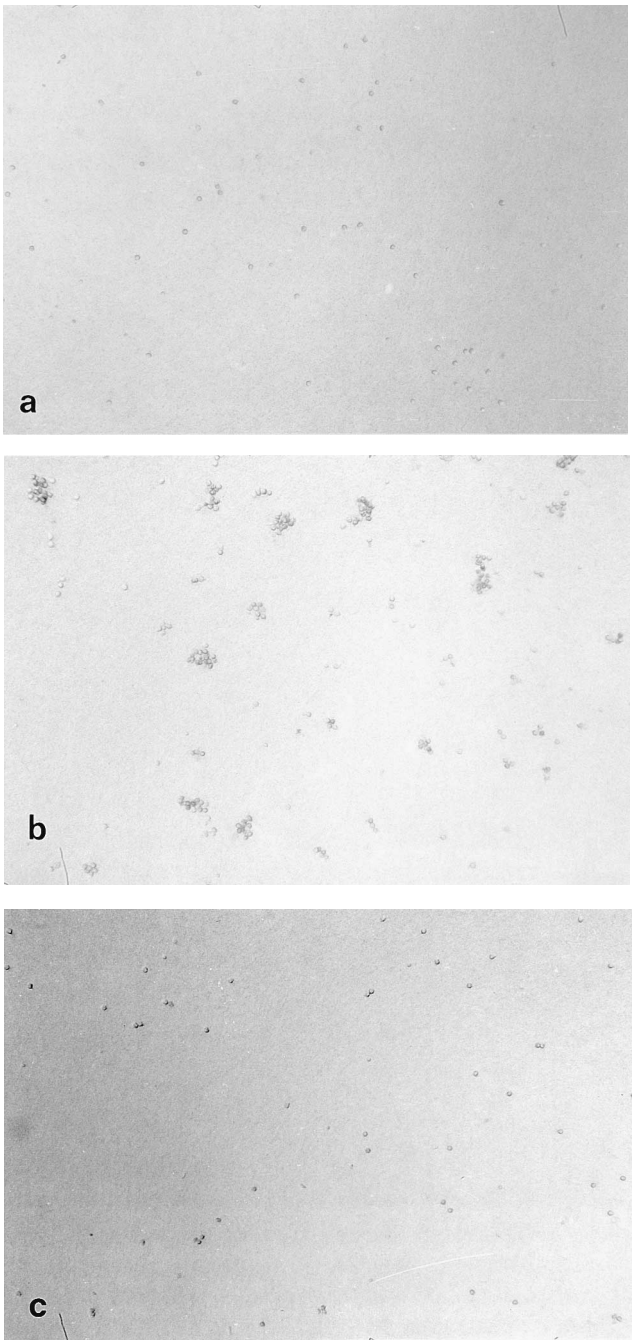


FIG. 1. NA and inhibition of NA. (a) PMNs alone in PBS, unagglutinated; (b) PMNs plus strain IH11128 (expressing the Dr hemagglutinin) in PBS, agglutinated; (c) PMNs plus strain IH11128 in PBS plus chloramphenicol, unagglutinated. Original magnification, ×100.

PMN adherence. PMN adherence assays confirmed the results of the NA assays, in that strains showed patterns of adherence consistent with the known activities of their respective adhesins (Fig. 2). Examples of fluorescence micrographs are presented in Fig. 3. BN406 (expressing the Dr adhesin) bound to PMNs (Fig. 3a), while EC901 (the corresponding host strain) showed much less PMN binding (Fig. 3b) and SH48 (expressing type 1 fimbriae) had greater numbers of PMN-bound organisms (Fig. 3c). When adherence was exam-

TABLE 3. Quantitative agglutination inhibition

Inhibitor[a]	IC_{50} (mM) for[b]:			
	Type 1 fimbriae[c]		Dr hemagglutinin[d]	
	HA	NA	HA	NA
D-Mannose	0.4	0.2	>450	>450
Methyl-α-D-mannoside	0.4	0.5	>450	>450
p-Nitrophenyl-α-D-mannoside	0.004	0.05	>1	>1
Chloramphenicol	>2	>2	0.02	0.04
BCBT	>1	>1	0.7	0.6

[a] Pigeon egg white and globoside failed to block HA and NA for both type 1 fimbriae and the Dr hemagglutinin.
[b] Final concentration of inhibitor in assays shown.
[c] Strain SH48.
[d] Strain IH11128.

ined quantitatively (Fig. 2), strains expressing adhesins of the Dr family (Dr, AFAI, AFAIII, and F1845) had similar numbers of adherent organisms (range, 4.0 to 7.92 organisms per PMN), whereas the recombinant strain expressing type 1 fimbriae bound PMNs to a much greater extent (16.18 organisms per PMN) and the recombinant strain expressing P fimbriae did not bind to PMNs.

Binding to PMNs was dependent on the size of the bacterial inoculum in the PMN-bacterial mixtures in a similar manner for strains bearing either type 1 fimbriae or the Dr hemagglutinin. As seen in Fig. 4, the relationships of adherence and inoculum were similar for these two organisms if the data are taken as a percentage of maximal binding. However, the total number of organisms bound per PMN at any given inoculum

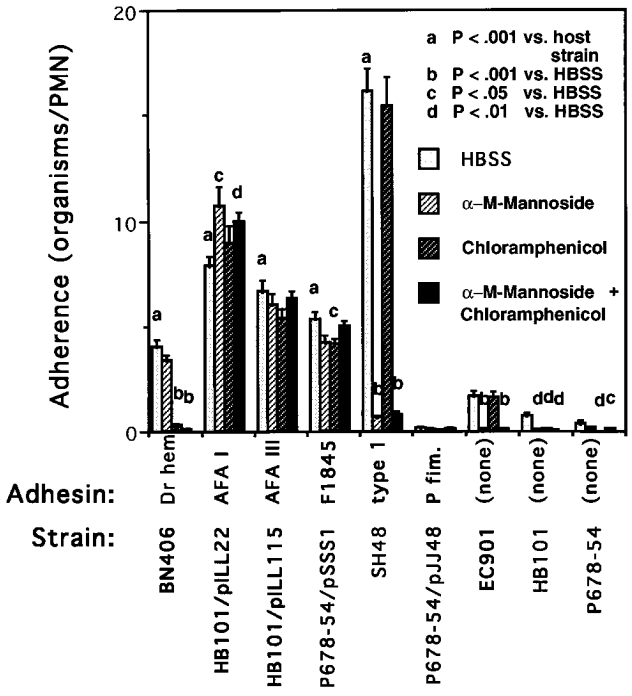


FIG. 2. Quantitative PMN adherence and adherence inhibition by α-methylmannoside (α-M-Mannoside) and chloramphenicol. Data are expressed as numbers of organisms bound per PMN (means ± standard errors). The bacterial inoculum was approximately 10^{10} organisms per ml. Adhesin, recombinant adhesin phenotype; Dr hem., the Dr hemagglutinin; F1845, F1845 fimbriae; type 1, type 1 fimbriae; P. fim., P fimbriae.

was about 5 times higher for the type 1-fimbriated strain than for the strain expressing Dr hemagglutinin. Binding of either strain to PMNs was not saturable at the highest inoculum tested (2×10^{10} to 3×10^{10} bacteria per ml, equivalent to a bacteria/PMN ratio of approximately 1,000:1).

Similarly, addition of inhibitors confirmed the results of the NA inhibition assays (Fig. 2), whereby adherence of the recombinant strain expressing type 1 fimbriae (SH48) was inhibited only (and almost completely) by mannose and adherence of the Dr hemagglutinin-expressing strain (BN406) was inhibited only (and almost completely) by chloramphenicol. An example of the latter can be seen in Fig. 3d. PMN adherence of the Dr hemagglutinin-bearing strain was inhibited by chloramphenicol in a concentration-dependent manner (Fig. 5), with an IC_{50} of 0.16 mM. PMN adherence of the strains expressing the other Dr family adhesins was not consistently inhibited by either chloramphenicol or mannose (Fig. 2). With PMNs from a patient with the severe phenotype of leukocyte adhesion deficiency of the type lacking the putative PMN receptor for type 1 fimbriae (40), adherence of the type 1-fimbriated strain SH48 was markedly reduced whereas there was no diminution of adherence with the Dr hemagglutinin-bearing strain BN406 (data not shown).

Adherence inhibition assays also revealed slight mannose-sensitive adherence to PMNs by host strains EC901 and HB101 (Fig. 2), with a relative intensity of adherence consistent with each strain's intensity of agglutination of *Saccharomyces cerevisiae* (Table 1). However, the intensity of mannose-sensitive PMN adherence for these strains was 10- to 20-fold lower than for the recombinant type 1-fimbriated strain SH48 (Fig. 2).

PMN microbicidal activity. To evaluate the functional consequences of adherence to PMNs mediated by the Dr hemagglutinin, we measured PMN microbicidal activity with the Dr recombinant stain BN406 in comparison with the corresponding host strain EC901, using the type 1-fimbriated recombinant strain SH48 (in conjunction with the corresponding host strain P678-54) as a positive control for adhesin-enhanced PMN microbicidal activity. As seen in Table 4, in the absence of serum opsonization the strain expressing type 1 fimbriae (SH48) was killed by PMNs to a much greater extent than the corresponding host strain (P678-54). In contrast, although there was a slight trend towards greater killing of Dr hemagglutinin-bearing organisms (BN406) than of those of the corresponding host strain (EC901), this difference was not statistically significant (Table 4). Opsonization with C8-deficient serum dramatically increased PMN-mediated killing of both Dr-positive and type 1-fimbriated organisms. Viability at 30 and 60 min declined from an initial 100% to 10.1 ± 4.3% and 2.4 ± 1.1%, respectively, for the Dr-adhesin-bearing strain BN406 and to 3.4 ± 0.6% and 0.5 ± 0.1% for the type 1-fimbriated strain SH48.

DISCUSSION

These findings clearly confirm our first hypothesis, that adhesins of the Dr family mediate adherence to and agglutination of PMNs. This Dr adhesin-mediated adherence and agglutination were not antagonized by inhibitors of other urinary tract infection-related *E. coli* adhesins (type 1 and P fimbriae) and was unaffected by the absence of the putative PMN receptor for type 1 fimbriae (40). In contrast, adherence to PMNs mediated by the Dr hemagglutinin (but not by other adhesins of the Dr family) was specifically antagonized by chloramphenicol and its analog BCBT, both of which are inhibitors of HA mediated by the Dr hemagglutinin (but not by other adhesins

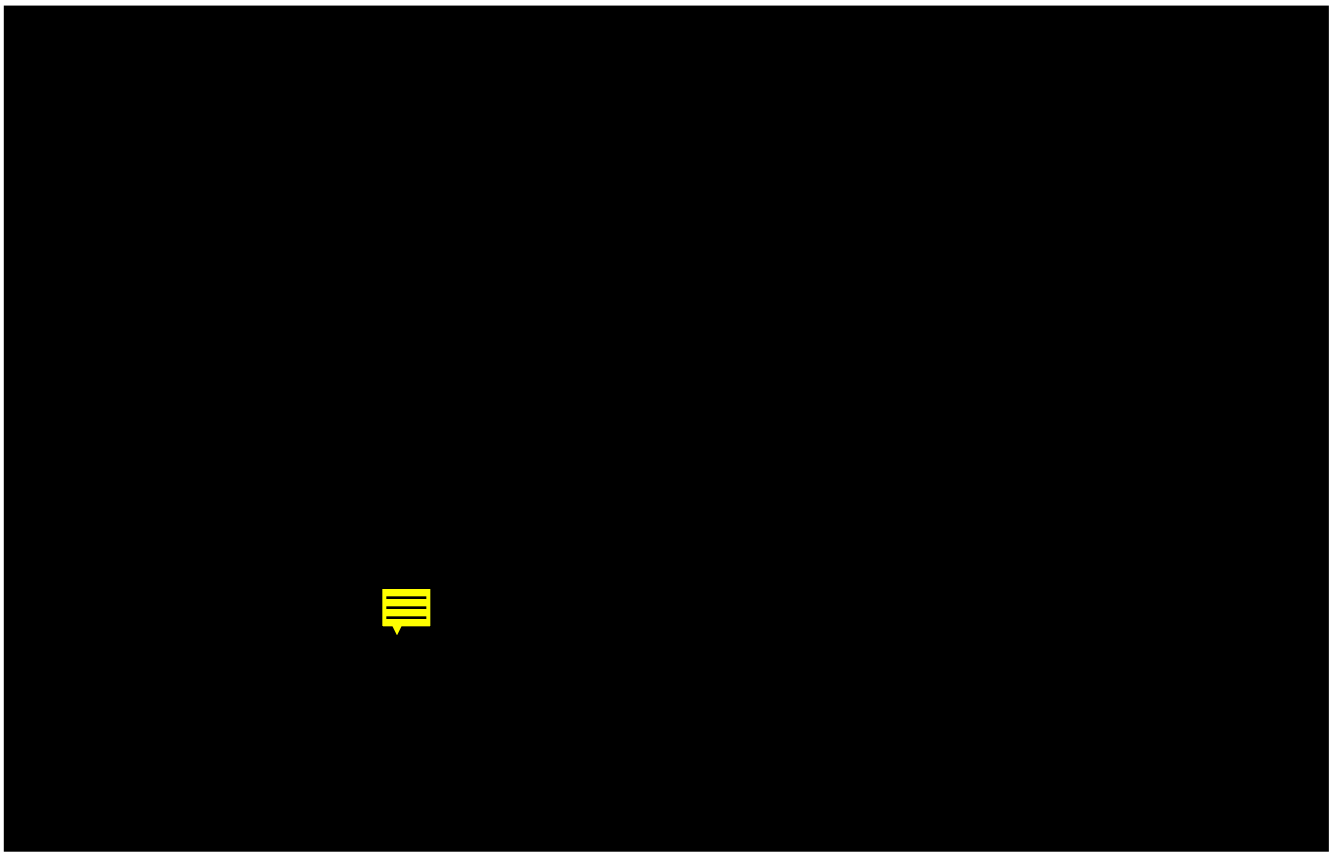


FIG. 3. PMN adherence. Representative fluorescence micrographs of bacterial binding to PMNs. (a) Strain BN406 (expressing the Dr hemagglutinin); (b) strain EC901 (the corresponding host strain); (c) strain SH48 (expressing type 1 fimbriae); (d) strain BN406 plus chloramphenicol. Original magnification, ×1,000.

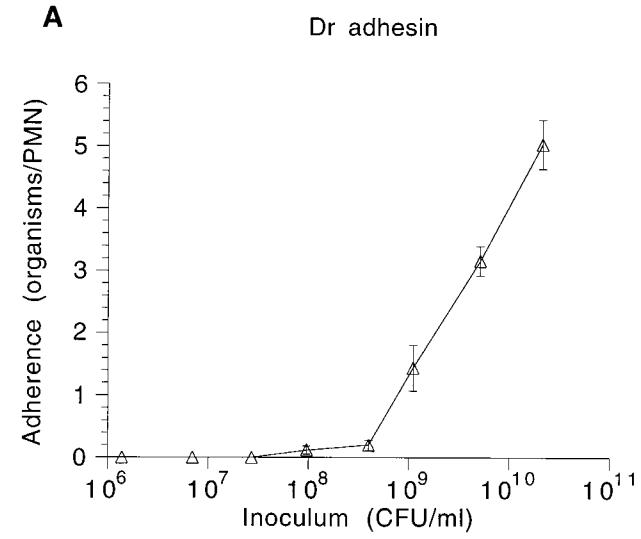
of the Dr family) (39), as confirmed here (Tables 2 and 3; and Fig. 2).

These observations, together with the similar concentrations of inhibitors required to block Dr adhesin-mediated adherence to PMNs and erythrocytes (Table 3; Fig. 5), suggest that the receptor on PMNs for the Dr hemagglutinin (and, by extension, the rest of the Dr family) is biochemically similar to the receptor for these adhesins on erythrocytes. This conclusion is consistent with the hypothesis that DAF, which is present on PMNs (31, 32) and is the Dr adhesin receptor on erythrocytes (39) and in human tissues (19), is the Dr adhesin receptor on PMNs. In particular, the short consensus repeat 3 (SCR3) domain of DAF has been shown to be the binding site for the entire Dr family of adhesins (34). The only known putative receptor for the Dr hemagglutinin other than DAF is type IV collagen (54); that this extracellular matrix molecule might be the relevant Dr adhesin receptor on PMNs is quite unlikely.

Our second hypothesis, i.e., that adherence to PMNs would have adverse consequences for Dr adhesin-bearing strains, was not supported by our data. Bacterial killing in the presence of PMNs was slightly but not significantly greater for the Dr hemagglutinin-expressing recombinant strain BN406 than for the corresponding host strain EC901 (Table 4). In contrast, killing of the type 1-fimbriated recombinant strain SH48 was dramatically greater than that of the corresponding non-type 1-fimbriated host strain P678-54 (Table 4). Furthermore, when PMN-bacterial interactions were examined by fluorescence microscopy, it appeared that most Dr adhesin-bearing organisms associated with PMNs remained extracellular whereas many type 1-fimbriated organisms associated with PMNs had been internalized (Fig. 3a and c). It is likely (but remains to be confirmed experimentally) that other members of the Dr adhesin family would give results similar to those we obtained with the Dr hemagglutinin, since despite some microheterogeneity of binding specificity all these adhesins recognize the same receptor molecule (DAF) on erythrocytes and, presumably, on PMNs.

These observations suggest that whereas expression of type 1 fimbriae may constitute a liability for *E. coli* strains in their encounters with phagocytes (17), the same may not be true for Dr family adhesins. The better survival of Dr adhesin-bearing strains than of type 1-fimbriated strains in in vitro killing assays done without serum may not reproduce in vivo situations in which bacteria are opsonized with complement, since PMN-mediated killing was markedly increased (and similar after serum opsonization) for both type 1-fimbriated and Dr adhesin-bearing strains. However, at complement-poor sites such as the urinary space and the renal medulla or with complement-resistant encapsulated wild-type strains, effective complement-mediated opsonization may not occur, sparing Dr adhesin-bearing strains the phagocytosis and killing by PMNs that their type 1-fimbriated counterparts suffer.

Phase variation is a well-recognized phenomenon with several adhesins of uropathogenic *E. coli*, including type 1, P, and S fimbriae (1, 9, 38, 43). For type 1 and S fimbriae, phase variation has been proposed as a mechanism whereby a fraction of the bacterial population (i.e., the adhesin-negative fraction) may avoid deleterious interactions with phagocytes (40).



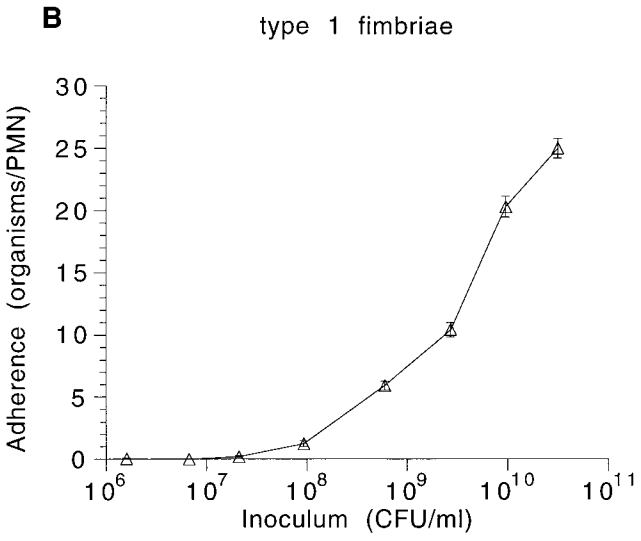


FIG. 4. Relationship between PMN adherence and bacterial inoculum. (A) Strain BN406 (expressing the Dr hemagglutinin); (B) strain SH48 (expressing type 1 fimbriae). Data are expressed as numbers of organisms bound per PMN (means ± standard errors).

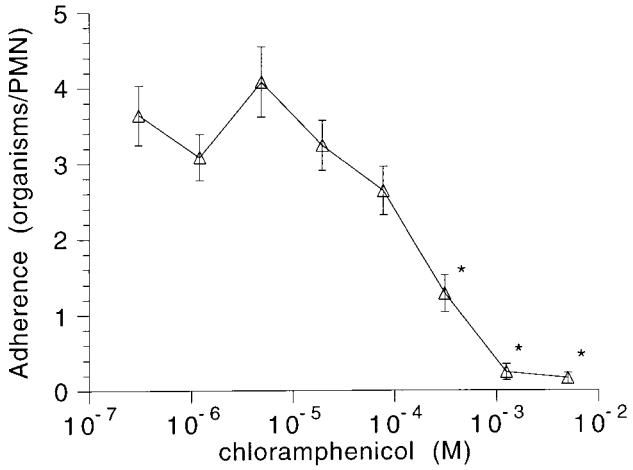


FIG. 5. Inhibition by chloramphenicol of adherence to PMNs by strain BN406 (expressing the Dr hemagglutinin). Assays were performed in the presence of 100 mM methyl-α-D-mannoside to eliminate mannose-sensitive adherence attributable to the host strain EC901 itself. Data are expressed as numbers of organisms bound per PMN (means ± standard errors). *, $P < 0.05$ versus adherence at lowest chloramphenicol concentration tested.

Our finding that Dr adhesin-mediated adherence to PMNs does not result in significantly increased bacterial killing suggests that avoidance of adherence to phagocytes may not provide a survival advantage to Dr adhesin-negative phase variants. However, by adhering to PMNs Dr-positive bacteria may be sequestered and prevented from participating in colonization and invasion, particularly if the PMNs are voided with the urine. Thus, phase variation may provide a persistence advantage in the urinary tract by allowing Dr adhesin-negative phase variants to avoid sequestration by PMNs.

Alternatively, at tissue sites such as the renal parenchyma where infiltration with PMNs occurs during acute infection, passive adherence to PMNs in the absence of killing may allow Dr-positive bacteria to hitchhike through tissues on migratory PMNs. In this role, Dr adhesins would constitute an invasion-promoting virulence factor, as would phase switching to an adhesin-negative state by allowing adherent bacteria to dissociate from PMNs and initiate tissue colonization at a new site.

The number of organisms bound per PMN varied dramatically depending on the recombinant adhesin expressed. Adherence values for the type 1-fimbriated strain SH48 were more than twice as high as those for any of the strains expressing Dr family adhesins. Interestingly, the AFAI-expressing strain had the highest level of binding amongst the Dr family; a similar finding was previously demonstrated with CHO cell transfectants expressing DAF on their surfaces (34). The explanation for this variability between strains bearing different adhesins in the number of organisms bound per PMN is unknown but could relate to quantitative differences in expression of the recombinant adhesins in the host *E. coli* strains, differences in binding affinities of the various adhesins for their corresponding receptors, or differences in the numbers or accessibility of receptors present on the surface of PMNs.

The slight inhibition by mannose of adherence to PMNs observed with the MR adhesin-negative host strains EC901 and HB101 (Fig. 2) can be explained by their weak to moderate expression of mannose-sensitive adhesins (Table 1). This mannose-sensitive adherence, although statistically significant, was of trivial magnitude and was greatly overshadowed in the

TABLE 4. Bacterial viability after exposure to PMNs

Strain	Recombinant adhesin	% Viability at indicated time of exposure (min)[a]	
		30	60
BN 406	Dr hemagglutinin	87.1 ± 10.7	65.7 ± 17.8
EC901	None	112.9 ± 26.5	80.7 ± 14.4
SH48	Type 1 fimbriae	19.9 ± 7.1[b]	10.8 ± 4.3[c]
P678-54	None	98.0 ± 12.0	43.4 ± 10.2

[a] Data are expressed as percents of initial viability (means ± standard errors; $n = 3$ to 6).
[b] $P < 0.001$ versus corresponding host strain.
[c] $P < 0.02$ versus corresponding host strain.

corresponding recombinant strains by adherence attributable to the cloned Dr family adhesins (Fig. 2). The slight decreases in adherence seen with chloramphenicol for strains HB101, P678-54, and the F1845-fimbriated recombinant strain P678-54/pSSS1, as well as the slight increase in adherence seen with mannose for the AFA1 recombinant strain HB101/pILL22 (Fig. 2), were unanticipated and are unexplained.

Adherence of *E. coli* to PMNs and other effector cells mediated by type 1 fimbriae and S fimbriae is known to result in a variety of responses from the host cells, including stimulation of the respiratory burst, release of granular contents and other mediators, and increased arachidonate metabolism (17, 52). These effects may result in host injury and promote the inflammatory response, thereby contributing to disease pathogenesis. Whether adherence to PMNs mediated by Dr family adhesins, as documented here, triggers similar (or other) responses from phagocytes remains to be determined.

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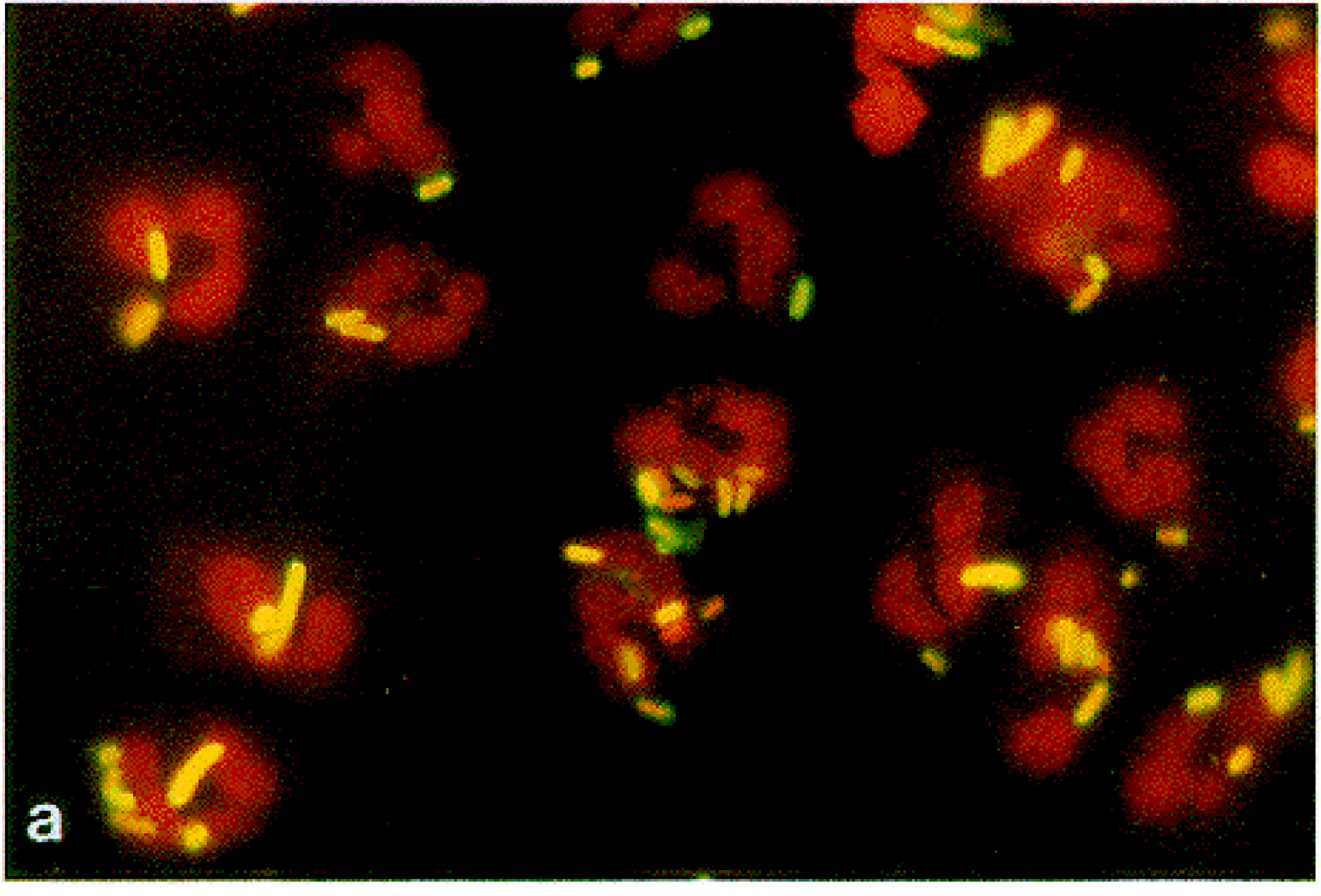
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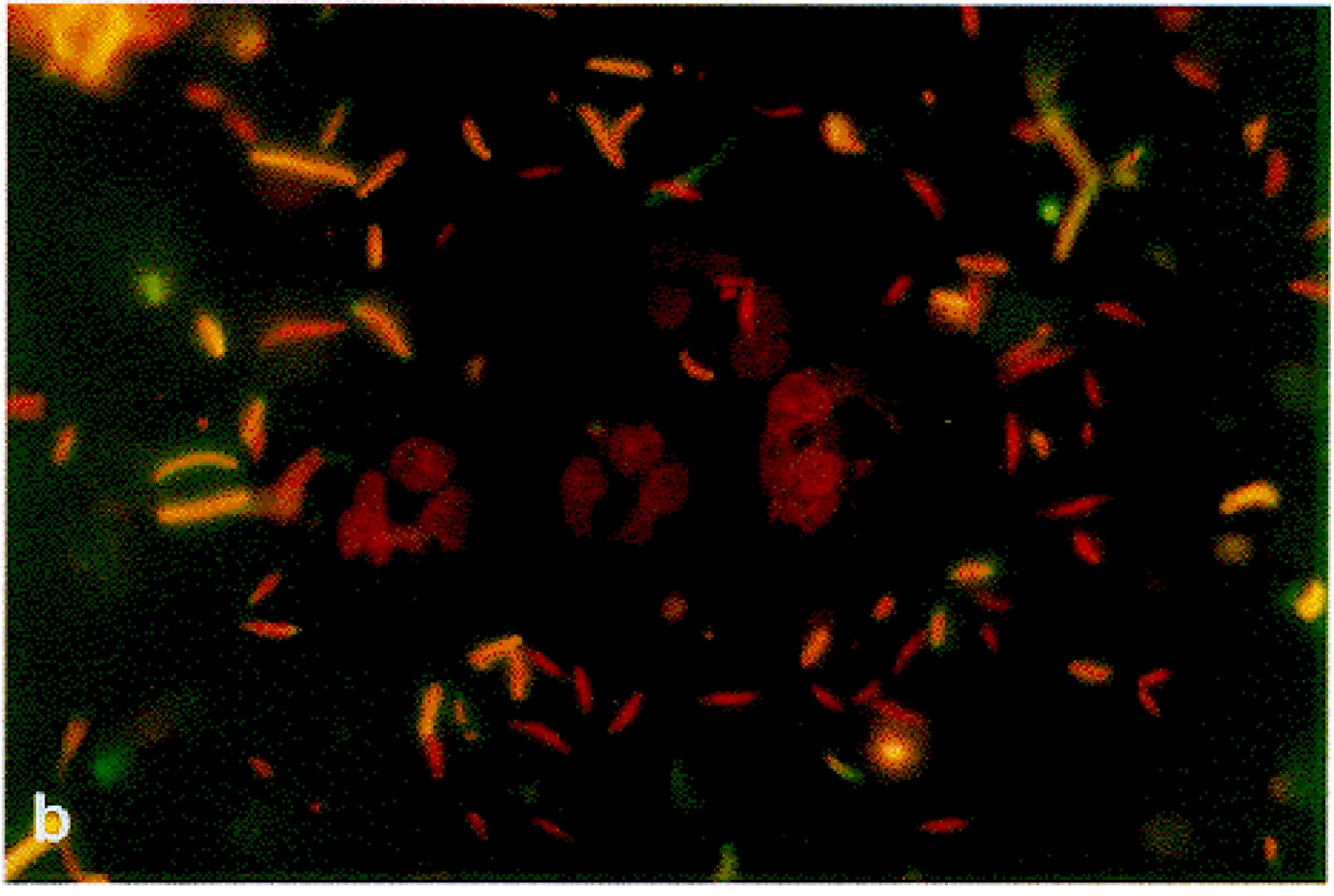
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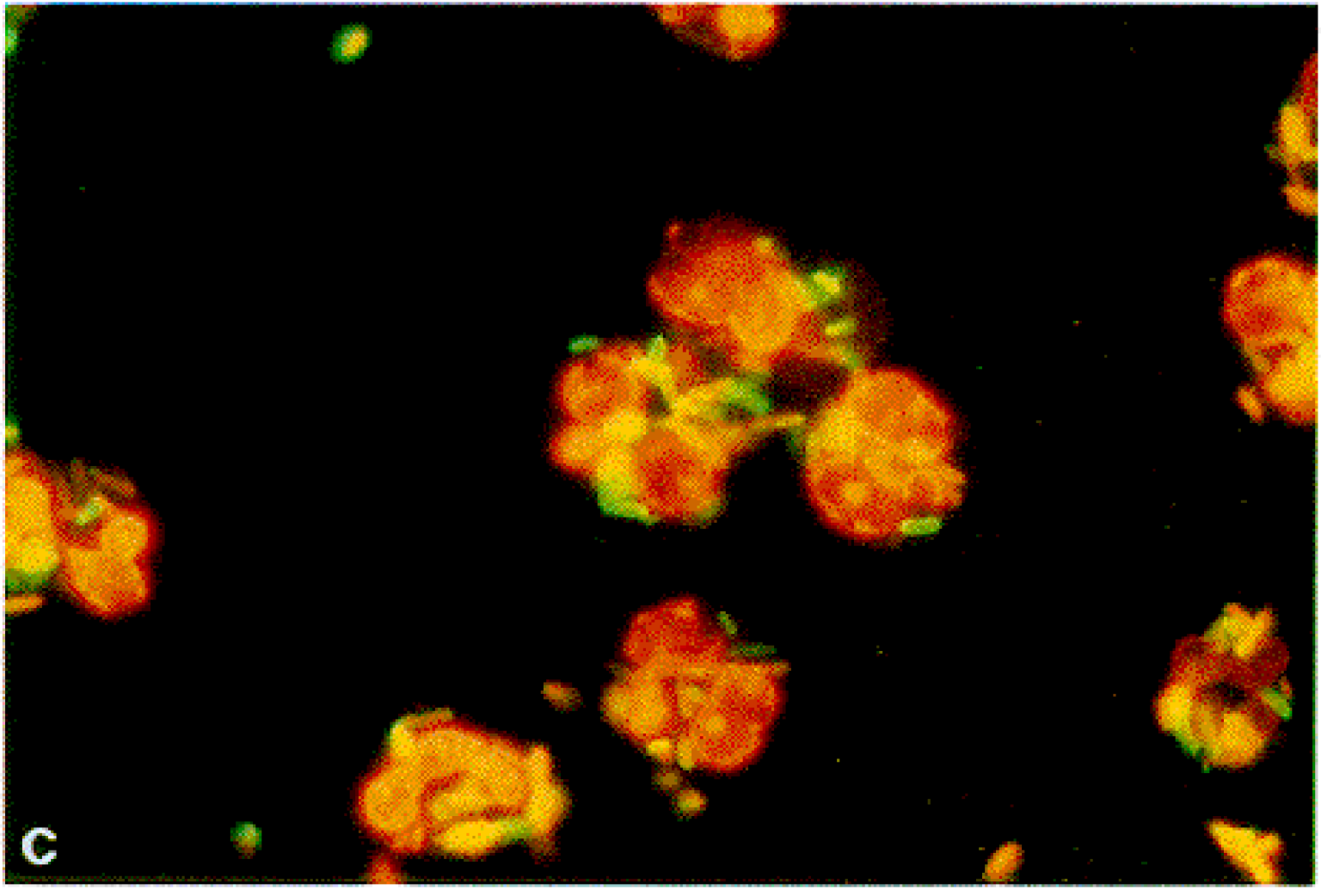
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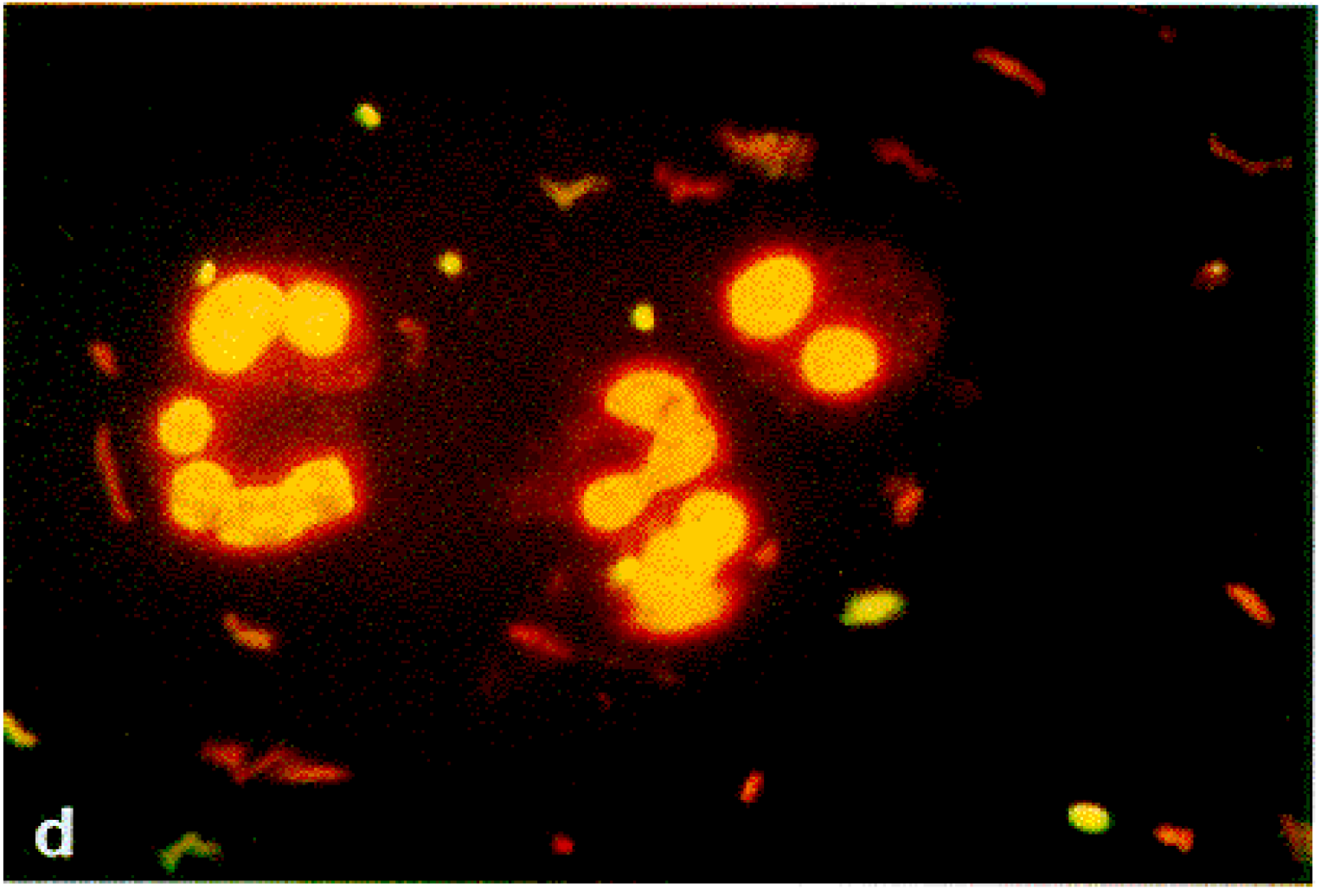
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