Monoclonal Antibodies to TEM-1 Plasmid-Mediated β -Lactamase

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At least 28 plasmid-mediated β -lactamases have been described in gram-negative bacteria. To assess the relationship among these enzymes, we produced and characterized 28 murine monoclonal antibodies to the TEM-1 plasmid-mediated β -lactamase. Radial immunodiffusion identified 3 monoclonal antibodies as immunoglobulin M (IgM), 18 as subclass IgG1, 2 as IgG2a, and 5 as IgG2b. Using a newly described enzyme immunoassay, cross-reactivity of 16 of these monoclonal antibodies was tested against 24 plasmid-determined β -lactamases. The 16 monoclonal antibodies cross-reacted with TEM-2 and TLE-1 and, to a certain extent, SHV-1. Different levels of cross-reactivity were also observed with OXA-3 (11 of 16), OXA-7 (8 of 16), OXA-1 (2 of 16), OXA-6 (2 of 16), and AER-1 (2 of 16). Six monoclonal antibodies demonstrated partial neutralization of β -lactamase activity. This study suggests that common epitopes are shared by nine biochemically distinct plasmid-mediated β -lactamases. On the basis of cross-reactivities with these monoclonal antibodies, we identified four epitopes on TEM-1, TEM-2, TLE-1, and SHV-1 β -lactamases.

Four mechanisms of bacterial resistance to β -lactam antibiotics have been described in gram-negative bacteria (13): (i) modification of cell-wall permeability (46), (ii) modification of penicillin-binding proteins (54), (iii) tolerance to the antibiotic (58), and (iv) enzymatic modification of the drug (34). Among these, the most frequent mechanism is the production of β -lactamases (EC 3.5.2.6) which hydrolyze the antibiotic, rendering it inactive (34). These enzymes are produced by almost all bacterial species and differ from each other in their enzymological and physicochemical properties. Although three classifications have been proposed (1, 42, 56), we tend to refer to β -lactamases as plasmid-mediated or chromosomal enzymes. At least 28 plasmid-mediated β -lactamases are known (5, 15, 19, 24, 26, 30, 31, 36, 38, 45, 53), including the newly described BRO-1 (11), NPS-1 (25), OHIO-1 (51), and SAR-1 (39). Of all these enzymes, the most widely distributed is the TEM-1 β -lactamase (26, 44, 52).

Three techniques are available to identify plasmid-mediated β -lactamases precisely: analytical isoelectric focusing (27), DNA hybridization (23), and immunological assays (40, 41). Several studies have reported immunological comparisons between plasmid-mediated β -lactamases using polyclonal antisera directed against TEM-1 (22, 28, 29, 35, 36, 38, 47-49, 57), SHV-1 (28, 57), OXA-1 (29, 36), OXA-2 (16), OXA-4 (37, 38), PSE-1 (29), PSE-2 (29, 57), PSE-4 (29), and CARB-3 (36, 38). Until now, immunological methods used to identify or classify β -lactamases were primarily based on the specific neutralization of the enzyme activity by polyclonal antisera. Monoclonal antibodies (MAbs) in combination with a more sensitive method, such as an enzyme immunoassay (EIA), have not been used to characterize plasmid-mediated β -lactamases even though the utility of MAbs has been described in many other areas (7, 20, 21, 60).

We prepared MAbs to the TEM-1 β -lactamase and tested their reactivity against 24 known plasmid-mediated β -lactamases. Using a newly described EIA (C. J. Morin, G. Richer, M. Couillard, and R. Letarte, Abstr. Annu. Meet. Am. Soc. Microbiol. 1984, A43, p. 8), immunological similarities were detected among nine of these enzymes. This

study suggests that there is a common epitope located on the latter group of nine plasmid-mediated β -lactamases.

MATERIALS AND METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used in this study are shown in Table 1. Although its gene is chromosomal, the properties of AER-1 β -lactamase (15) closely resemble those of other CARB-type enzymes so that with this qualification, all the β -lactamases listed in Table 1, including transposable β -lactamases, are referred to as plasmid mediated.

β -Lactamase preparation. Bacterial strains were inoculated into Trypticase soy broth (BBL Microbiology Systems, Cockeysville, Md.) and incubated at 37°C for 18 h. Cells were harvested, washed once with 85 mM NaCl, and suspended at a density of 10% (wt/vol) in 100 mM potassium phosphate buffer (pH 7.0). Crude extracts were prepared by sonication (Sonic 300 Dismembrator; Artek Systems Corp., Farmingdale, N.Y.) twice for 4 min at a power of 180 W. Cell membranes were removed by centrifugation at 31,300 $\times$ g for 20 min, and the extracts were stored at -70°C until used. TEM-1 β -lactamase was purified by affinity chromatography on phenylboronic acid-agarose using a hydrophobic spacer arm (type-B column) as described elsewhere (8). Purified enzyme (420 μ g) was obtained with specific activity of the preparation equal to 110 U/mg of protein. The factor of purification was 30 $\times$, and the yield was 58%. One unit (U) is defined as the amount of β -lactamase which hydrolyzes 1 μ mol of pyridinium-2-azo-*p*-dimethylaniline chromophore (PADAC; Calbiochem-Behring, La Jolla, Calif.) (50) per min at 25°C (pH 7.0).

Preparation of specific MAbs. Repeated attempts to immunize BALB/c mice, the conventional strain used in the preparation of MAbs, were unsuccessful. Therefore, we immunized different strains of mice and found that TEM-1 was most immunogenic in CB6F1 mice. Hence, for fusion purposes, CB6F1 mice were immunized intraperitoneally with 15 μ g of purified TEM-1 β -lactamase emulsified in complete Freund adjuvant. At 2-week intervals for a period of 6 weeks, similar injections were given in incomplete adjuvant. Three and two days before the fusion, booster doses were given intravenously. Spleen cells of a hyperim-

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TABLE 1. Bacterial strains and plasmids

Plasmid ^a	β -Lactamase	Reference
pBR322	TEM-1	55
pMK20::TnI	TEM-2	18
pMG204b	TLE-1	30
R997	HMS-1	28
R974	SHV-1	28
pMON80	LCR-1	23
pMON401	ROB-1	23
RGN238	OXA-1	59
R46	OXA-2	2
pMON916	OXA-3	23
pMON400	OXA-4	23
pMON53	OXA-5	23
pUZ8-pMG39	OXA-6	30
pMG202	OXA-7	30
pMON810	PSE-1	23
pMON230	PSE-2	23
pMON225	PSE-3	23
pMON705	PSE-4	23
pMON41	CARB-3	23
pMON1025	CARB-4	23
pMON510	AER-1	23
pNU81	CEP-1	19
pLQ3	CEP-2	24
R22Ka	CEP-like	5

^a Plasmids were carried by one of the following host cells: *Escherichia coli* C600 F⁻ *lacY leu thi thr*, *E. coli* HB101 F⁻ *lacY leu pro thi recA rpsL20*, or *E. coli* J53-2 F⁻ *met pro Rif*^r (4, 6, 14).

mune mouse were fused with myeloma NSO cells as previously described (12). A total of 560 hybridomas were recovered, of which 171 were initially positive by at least one of the EIAs. Twenty-eight of these were successfully cloned by limiting dilution. Culture supernatants of the same MAbs were pooled, frozen, and used as a standard source of MAbs for all the experiments.

Identification of immunoglobulin classes. The classes and subclasses of monoclonal immunoglobulins were determined using radial immunodiffusion in 1% agarose dissolved in phosphate-buffered saline (PBS). Undiluted rabbit anti-mouse antibodies (Bionetics Laboratory Products, Kensington, Md.) specific for immunoglobulin M (IgM), IgG1, IgG2a, IgG2b, IgG3, and IgA and antibodies specific for κ and λ light chains were placed in the central well, and the MAbs were placed in the surrounding wells. The plates were placed in a humid chamber at room temperature for 24 h and then at 4°C for 48 h. The precipitation bands were then visualized using Coomassie brilliant blue (Bio-Rad Laboratories, Richmond, Calif.).

EIAs. To prevent the possibility of missing interesting hybridomas, two EIAs were used to evaluate MAb production: a horseradish peroxidase EIA (EIA-HRP) and a PADAC EIA (EIA-PADAC). In the EIA-HRP (Fig. 1), the wells of polyvinyl chloride microtitration plates (Falcon; Becton Dickinson Labware, Oxnard, Calif.) were coated overnight at 4°C with 10 ng of purified TEM-1 β -lactamase in 100 μ l of PBS (20 mM)-0.02% sodium azide (PBSA) (pH 7.2). Uncoated plastic was then saturated by adding 100 μ l of PBSA-bovine serum albumin (6%) to the wells. After an incubation of 3 h at room temperature, the plates were washed three times with PBS containing 0.05% Tween 20 (PBST), and 100 μ l of hybridoma culture supernatants diluted 1/3 in PBST was placed in wells. Preimmune and

hyperimmune mouse anti-TEM-1 antisera diluted 1/1,000 were used, respectively, as negative and positive controls. The plates were left at room temperature for 3 h. They were then washed four times with PBST and filled with 100 μ l of a 1/1,000 dilution in PBST of peroxidase-conjugated rabbit anti-mouse immunoglobulins (RAMIG-HRP; Dako, Copenhagen, Denmark). After 3 h of incubation at room temperature, the plates were washed six times with PBST, and 100 μ l of 2,2'-azino-bis(3-ethylbenzthiazoline sulfonic acid) (ABTS; Sigma Chemical Co., St. Louis, Mo.) (0.04%)-H₂O₂ (0.012%) in citrate-phosphate buffer (73 mM; pH 4.5) was added in the wells. Optical densities (ODs) were recorded after 45 min at room temperature by a Titertek Multiskan MC plate spectrophotometer (Flow Laboratories, Irvine, Scotland) at 414 nm.

In the EIA-PADAC (Fig. 2), polyvinyl chloride plates were coated with 100 μ l of a 1,000-fold dilution in PBSA of rabbit anti-mouse immunoglobulins (RAMIG; Dako) and incubated overnight at 37°C. Uncoated plastic was then saturated with bovine serum albumin as in the EIA-HRP. The plates were then washed three times with PBST, and hybridoma culture supernatants were added as in the EIA-HRP and incubated for 3 h at room temperature. As determined by checkerboard titration using a standard sandwich technique with RAMIG-HRP at stage 3, unlabeled RAMIG used for stage 1 was completely saturated with each of the 28 tested mouse MAbs (stage 2) at the dilution used for the

FIG. 1. Schematic representation of the steps involved in the EIA-HRP. BSA, Bovine serum albumin.

assay. The plates were then washed four times with PBST, and 100 μ l of PBST containing 1 mU of TEM-1 β -lactamase was added. The same amount (1 mU) was used for all β -lactamases when cross-reactivities were tested. After an incubation of 3 h at room temperature, the plates were washed six times with PBST and 100 μ l of PADAC (140 μ M) in phosphate buffer (100 mM; pH 7.0) was added in each well. ODs were recorded with the plate spectrophotometer at 540 nm after 45 min and 16 h at room temperature.

For the two EIAs, control wells missing either one of the three stages were always included in all experiments for all different MABs and all different β -lactamases.

Enzyme neutralization assay. Using microtitration plates, 25 μ l of PBS containing 1 mU of crude β -lactamase was mixed with 25 μ l of undiluted hybridoma culture supernatants and placed at 37°C for 1 h. PADAC (50 μ l; 280 μ M) was then added, and ODs were measured after 2, 5, 10, 30, and 60 min with the plate spectrophotometer at 540 nm. Neutralization percentage was determined by comparing these values with those obtained with controls (0% neutralization) in which hybridoma culture supernatants had been replaced by fresh culture medium.

RESULTS

Preparation and characterization of specific MABs. Of the 560 hybridomas recovered after the fusion, 171 positive

FIG. 2. Schematic representation of the steps involved in the EIA-PADAC. BSA, Bovine serum albumin.

TABLE 2. Reactivity of anti-TEM-1 MABs in EIA-HRP and EIA-PADAC and neutralization of TEM-1 β -lactamase activity

Hybridoma	Class ^a	EIA-HRP ^b	EIA-PADAC ^c	Neutr. (%) ^d
MOC1	IgG2b (κ)	+	—	
MOC2	IgM (κ)	+	—	
MOC3	IgG1 (κ)	+	+	
MOC4	IgM (κ)	+	—	
MOC5	IgG1 (λ)	—	+	
MOC6	IgG1 (λ)	—	+	
MOC7	IgG1 (κ)	+	—	
MOC8	IgG2b (κ)	+	+	81
MOC9	IgG1 (κ)	+	—	
MOC10	IgG2b (κ)	+	—	
MOC11	IgG2a (κ)	+	+	84
MOC12	IgG1 (κ)	+	—	
MOC13	IgM (κ)	+	—	
MOC14	IgG1 (κ)	+	+	
MOC15	IgG1 (κ)	+	+	86
MOC16	IgG1 (λ)	+	+	
MOC17	IgG1 (κ)	+	—	
MOC18	IgG2b (κ)	+	—	
MOC19	IgG1 (κ)	+	+	
MOC20	IgG1 (κ)	+	+	90
MOC21	IgG1 (κ)	+	+	
MOC22	IgG1 (κ)	+	—	
MOC23	IgG1 (κ)	+	+	
MOC24	IgG1 (κ)	+	—	
MOC25	IgG1 (κ)	+	+	89
MOC26	IgG2a (κ)	+	+	
MOC27	IgG1 (κ)	+	+	88
MOC28	IgG2b (λ)	—	+	

^a κ , κ light chains; λ , λ light chains.

^b +, OD of >0.400 at 414 nm as measured by Multiskan MC spectrophotometer; —, OD of $\leq$ 0.400.

^c +, OD of <1.513 at 540 nm as measured by Multiskan MC spectrophotometer; —, OD of $\geq$ 1.513.

^d Neutr., Neutralization of β -lactamase activity compared with activity without MAB (blank space, 0% [no neutralization]).

clones were transferred in 24-well cluster trays. All the positive clones were grown for 1 week, and 45 of the 150 still-positive clones were chosen for cloning by limiting dilution. Twenty-eight of the cloned hybridomas, designated MOC1 to MOC28, were subcultivated and stored in liquid nitrogen. Twelve of these were positive by the EIA-HRP alone, three were positive by the EIA-PADAC alone, and thirteen were positive by both EIAs. Six of the latter also showed partial neutralization of enzyme activity (Table 2). As determined by radial immunodiffusion, 3 MABs were found to belong to the IgM class, 18 belonged to the IgG1 subclass, 2 belonged to the IgG2a subclass, and 5 belonged to the IgG2b subclass. Also, 24 MABs were found to be composed of κ light chains, and the other 4 were composed of the λ type (Table 2).

Cross-reactivity with other plasmid-mediated β -lactamases. Cross-reactivity of the 28 MABs was tested against 24 plasmid-mediated β -lactamases (Table 3). Since the EIA-HRP requires purified β -lactamases to coat the polyvinyl chloride plates, only the EIA-PADAC was used for this assay. Results are expressed on a scale of 0 to 9. OD values between 1.891 and 1.703 were recorded as 0 (OD, 1.891 for nonhydrolyzed PADAC), and OD values ranging from 0.189 to 0 were recorded as 9 (OD, 0 for totally hydrolyzed PADAC). Based on repeated experiments, including three final assays, results equal to or greater than 2 were considered positive. After 45 min of contact with PADAC, only 11 MABs (making epitope groups A, B, and C) visibly reacted with TEM-1, TEM-2, and TLE-1; 6 (epitope group B) of

TABLE 3. Cross-reactivity of anti-TEM-1 MAb with β -lactamases as determined by EIA-PADAC

Group ^a	Enzyme	Results ^b with MAb (MOC) ^c															
		3	5	6	8	11	14	15	16	19	20	21	23	25	26	27	28
I	TEM-1	9	8	9	9	9	5	9	9	9	9	4	9	9	9	9	8
	TEM-2	9	7	8	9	9	4	9	9	9	9	3	9	9	9	9	6
	TLE-1	9	8	8	9	9	5	9	9	9	9	4	9	9	9	9	7
	HMS-1	1	0	0	0	0	0	1	0	0	1	0	1	0	0	1	0
	SHV-1	4	2	2	9	9	3	9	3	3	9	3	4	9	4	9	2
	LCR-1	1	0	0	1	0	0	0	0	0	1	0	0	0	0	1	0
	ROB-1	2	3	2	2	2	2	2	2	3	2	2	2	2	2	2	2
II	OXA-1	2	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0
	OXA-2	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
	OXA-3	9	0	0	9	9	0	9	4	4	9	0	9	9	3	9	0
	OXA-4	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0
	OXA-5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	OXA-6	3	0	0	0	0	0	1	0	0	1	0	4	0	0	0	0
	OXA-7	7	0	0	2	2	0	4	1	1	4	0	7	3	1	4	0
III	PSE-1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	PSE-2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	PSE-3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	PSE-4	0	0	0	1	1	0	1	0	0	0	0	1	1	0	0	0
	CARB-3	0	0	0	0	0	0	1	0	0	0	0	1	1	0	0	0
	CARB-4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	AER-1	4	0	0	0	1	0	1	0	0	0	0	5	0	0	1	0

^a Groups refer to β -lactamase types. I, Broad spectrum (TEM-type); II, oxacillinases; III, carbenicillinases.

^b Results, obtained after an incubation of 16 h, are expressed on a scale of 0 to 9. 0, OD values from 1.891 to 1.703, and 9, OD values from 0.189 to 0, as measured by the Multiskan MC spectrophotometer at 540 nm. All results for group IV, cephalosporinases (CEP-1, CEP-2, and CEP-like), and host bacterial strains (controls; see footnote to Table 1) were 0.

^c Only those positive by the EIA-PADAC are listed, although all the MABs were tested. Those which are not listed gave 0, except with ROB-1, for which they gave 2. As explained in the text, the reactivity with ROB-1 was considered nonspecific.

these 11 MABs, in addition to the 3 preceding β -lactamases, reacted with SHV-1 and OXA-3; and 2 others (epitope group A) reacted with OXA-3, in addition to the 3 mentioned above. Maximum reaction was obtained only after 16 h of incubation; the results are shown in Table 3.

As expected, only the 16 hybridomas which were positive during the selection by the EIA-PADAC technique reacted with TEM-1. The other 12 did not react with TEM-1 or with any other β -lactamase in EIA-PADAC (they reacted only in the EIA-HRP) and therefore are not included in Table 3.

It can be seen that all the broad-spectrum β -lactamases classified in group I did not react with all the MABs. Only TEM-1, TEM-2, TLE-1, and SHV-1 reacted with all 16 MABs. HMS-1 and LCR-1 did not react with any. Even though significant values were obtained with ROB-1, cross-reactivity was considered negative, because this enzyme bound nonspecifically to bovine serum albumin-saturated polyvinyl chloride wells, giving identical results for control wells without MABs.

Of the group II β -lactamases, OXA-3 was the most reactive, i.e., it could be detected by 11 of the 16 MABs. OXA-7, OXA-6, and OXA-1 reacted with eight, two, and two MABs, respectively. OXA-2, OXA-4, and OXA-5 did not react with any.

Of the group III β -lactamases, only AER-1 reacted with two of the MABs (MOC3 and MOC23). None of the β -lactamases in group IV (CEP-1, CEP-2, and CEP-like) reacted with any of the 16 MABs.

On the basis of these reactivities, we can presume that there are at least four epitopes on the TEM-1 β -lactamase (Table 4). These four epitopes, named A through D, are shared by TEM-1, TEM-2, TLE-1, and SHV-1. OXA-3 has three of these four epitopes (A, B, and C); OXA-7 has two (A and B); and OXA-1, OXA-6, and AER-1 share only one (A).

Because the remaining β -lactamases did not react with any of the MABs, these enzymes do not share any of these four epitopes.

DISCUSSION

Anti-TEM polyclonal antibodies were first produced between 1968 and 1974 (17, 22, 47). Using such antibodies, many workers have tried immunological methods to detect, identify, or compare plasmid-mediated or chromosomal β -lactamases (16, 28, 35, 36, 38, 40, 41, 49, 57). Except for one report dealing with species-specific chromosomal β -lactamase of *Pseudomonas aeruginosa* (32), MABs have not been developed. In the present study, we report, for the first time, the isolation and characterization of 28 MABs directed against the plasmid-mediated TEM-1 β -lactamase.

The 28 MAB-producing hybridomas included in this study were selected using two distinct EIAs. As mentioned above, 12 of these hybridomas were positive by the EIA-HRP alone, 3 were positive by the EIA-PADAC alone, and 13 were positive by both EIAs. When the 28 MABs were tested afterwards for enzyme neutralization potency, only 6 neutralized the TEM-1 β -lactamase. These six neutralizing MABs make up the group recognizing epitope B. We did not observe any significant enhancement of the enzyme activity as previously reported (9, 32). Since the EIA-PADAC technique involves measuring the β -lactamase activity, it is surprising to find that the six neutralizing MABs were positive by both EIAs. Besides, it was to prevent this possibility of missing neutralizing MAB-secreting hybridomas that the EIA-HRP was performed simultaneously when screening. The long incubation period (16 h) does not explain this paradox, since all of these 6 neutralizing MABs were from the 11 early positives (after 45 min). The reversi-

TABLE 4. Grouping of MABs based on immunological reactivity with β -lactamases

Epitope group ^a	MAB (MOC) ^b	Reactions with β -lactamase ^c								
		TEM-1	TEM-2	TLE-1	SHV-1	OXA-1	OXA-3	OXA-6	OXA-7	AER-1
A	3	+	+	+	±	±	+	±	+	±
	23	+	+	+	±	±	+	±	+	±
B	8	+	+	+	+	—	+	—	±	—
	11	+	+	+	+	—	+	—	±	—
	15	+	+	+	+	—	+	—	±	—
	20	+	+	+	+	—	+	—	±	—
	25	+	+	+	+	—	+	—	±	—
	27	+	+	+	+	—	+	—	±	—
C	16	+	+	+	±	—	±	—	—	—
	19	+	+	+	±	—	±	—	—	—
	26	+	+	+	±	—	±	—	—	—
D	5	+	+	+	±	—	—	—	—	—
	6	+	+	+	±	—	—	—	—	—
	14	+	+	+	±	—	—	—	—	—
	21	+	+	+	±	—	—	—	—	—
	28	+	+	+	±	—	—	—	—	—

^a Based on the reactions observed in the EIA-PADAC.^b Only the 16 that reacted in the EIA-PADAC are listed.^c Only the nine that reacted specifically in the EIA-PADAC are listed. See Table 3. +, >5 results; ±, ≥2 to ≤5 results; —, <2 results.

bility of the antigen-antibody reaction combined with the high affinity of β -lactamases for PADAC (low K_m ; data not shown) and the fact that the neutralizing effect is not complete may explain why neutralizing MABs showed positive reactions by the EIA-PADAC. It is also possible that a given MAB fixed on a solid phase (EIA-PADAC) may not exhibit its ability to neutralize enzyme activity compared with unbound antibody in solution (neutralization).

Cross-reactions of the 16 anti-TEM-1 MABs with TEM-2 and TLE-1 and, to a certain extent, SHV-1 were foreseeable. It is well-known that the only structural distinction between TEM-1 and TEM-2 β -lactamases is a lysine amino acid (AAG codon) instead of a glutamine (CAG codon) at position 14 of the mature protein (55). Also, many researchers have reported either cross-reactivity of polyclonal antibodies between TEM-1 and TEM-2 β -lactamases (28, 29, 36, 48, 49, 57) or DNA cross-hybridization (10, 23). TLE-1 resembles TEM-1 in substrate profile and reactions with inhibitors (30). Moreover, a recent study (23) showed that a TEM-1 DNA probe cross-hybridizes with the TLE-1 gene, while previous attempts failed to demonstrate cross-hybridization (30). Based on enzymological data, resemblance between SHV-1 and TEM-1 is also well documented (26, 29). However, there is disagreement for immunological relatedness between these two TEM-type β -lactamases. Some workers have reported cross-reaction between these two enzymes (29), and some have found no cross-reaction (22, 28, 35, 43, 57). We have shown, especially for epitope B, that there is an immunological relationship between TEM-1 and SHV-1.

Cross-reactivity with OXA-1, OXA-3, OXA-6, OXA-7, and AER-1 was unexpected, because they have substrate and inhibition profiles that are different from those of the TEM-1 β -lactamase. In previous studies, polyclonal anti-TEM-1 antisera reactions with these enzymes were not tested or negative, probably due to the lack of sensitivity of the methods used. Moreover, no reaction was detected between TEM-1 and OXA-1 using specific TEM-1 and OXA-1 probes by DNA hybridization (23). On the other hand, cross-hybridization using an OXA-1 probe (23) and immunological cross-reaction using a polyclonal anti-OXA-4

antiserum (37) were reported between OXA-1 and OXA-4. We did not detect any positive reaction with OXA-4, while MOC3 and MOC23 (epitope A) reacted with OXA-1. These differences may be explained by speculating that in some regions of these proteins, the same amino acids coded by different codons are responsible for similar epitopes found on these β -lactamases. It may also be speculated that a similar epitope founded on TEM-1 and some of the OXAs is more immunogenic on TEM-1 than on OXA β -lactamases and that a similar epitope(s) between OXA-1 and OXA-4 is not the same as the epitope common to TEM-1 and OXA-1.

MABs have been used to differentiate related proteins in other areas (7, 20, 21, 60). However, the observed cross-reactivity may limit the use of anti-TEM-1 MABs for the identification of a given plasmid-mediated β -lactamase. Nevertheless, MABs bring new interest in view of the phylogenetical comparison of β -lactamases. On an evolutionary basis, it will be important to evaluate the activity of these MABs against different chromosomal β -lactamases. In this perspective, it will be interesting to compare their reactivities with different chromosomal β -lactamases, namely those of *Klebsiella* spp., since it has been postulated that TEM-1 β -lactamase may originate from *K. pneumoniae* (3, 29, 47–49) and SHV-1 may originate from *K. aerogenes* (33).

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