Elevations of Amniotic Fluid Macrophage Inflammatory Protein-1α Concentrations in Women During Term and Preterm Labor

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Objective: **To determine whether elevated concentrations of macrophage inflammatory protein-1α (MIP-1α) in amniotic fluid (AF) are related to term and preterm labor.**

Methods: **Amniotic fluid was obtained from women from five different clinical situations: 1) term cesarean delivery, no labor (n = 29); 2) normal term labor, no infection (n = 36); 3) preterm labor, delivery more than 1 week from sampling, no infection (n = 19); 4) preterm labor, delivery within 1 week from sampling, no infection (n = 18); and 5) preterm chorioamnionitis (n = 8). Amniotic fluid was collected aseptically at the time of amniocentesis, amniotomy, or hysterotomy. Concentrations of MIP-1α were determined by enzyme-linked immunosorbent assay. Statistical analysis was by Wilcoxon rank-sum test, Kruskal-Wallis test, and unpaired t test.**

Results: **Women in normal term labor had significant elevations of AF MIP-1α concentrations when compared with women at term undergoing repeat cesarean delivery (P < .001). In women with term gestation, AF MIP-1α correlated well with cervical dilation (r^2 = 0.479, P < .001). In women with preterm labor who later delivered within 1 week of presentation, AF MIP-1α concentrations were higher than those from women who did not deliver within 1 week. Women who presented with clinically evident chorioamnionitis had the highest concentrations of AF MIP-1α (P = .001).**

Conclusion: **Women in labor have significantly elevated AF concentrations of MIP-1α, particularly if labor is associated with intrauterine infection. We suggest that MIP-1α is involved in the physiology of normal labor and in the pathogenesis of infection-associated preterm labor.** *(Obstet Gynecol 1996;87:94–8)*

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Supported by a Clinical Investigator Award to DJD (KO8-HD00964) from the National Institutes of Health.

The pathophysiology of preterm labor remains incompletely understood, but 10–30% is likely due to subclinical intrauterine infection.[1] There are elevated levels of inflammatory cytokines in the amniotic fluid (AF) from women with infection-associated preterm labor. These include interleukin (IL)-1β,[2] tumor necrosis factor-α (TNF-α),[3] IL-6,[4–6] and IL-8.[7] We have shown[8–10] that human gestational tissues in culture produce IL-6 and IL-8 in response to stimulation with IL-1β, TNF-α, and lipopolysaccharide and are potential sources for these cytokines in AF. These cytokines play a key role in the inflammatory response to bacterial stimuli in gestational tissues and can mediate uterine activity by stimulating the production of uterotonic arachidonic acid metabolites, such as prostaglandin (PG) E_2 and 5-HETE.[11,12]

Another cytokine in the same family as IL-8 is macrophage inflammatory protein-1α (MIP-1α), which attracts monocytes, macrophages, and other immune effector cells into tissues and amplifies inflammatory responses.[13] The purpose of this study was to determine whether MIP-1α can be detected in the AF of women with normal term labor, preterm labor, and preterm chorioamnionitis.

Materials and Methods

Women with one of five clinical conditions were entered into this study: 1) term cesarean delivery, no labor (n = 29); 2) normal term labor, no infection (n = 36); 3) preterm labor, with delivery occurring more than 1 week from sampling and no clinically evident infection (n = 19); 4) preterm labor, with delivery occurring within 1 week from sampling and no clinically evident infection (n = 18); and 5) preterm chorioamnionitis (n = 8). Women who delivered within 1 week of presentation were assumed to be in preterm labor, whereas

 0029-7844/96/$15.00
SSDI 0029-7844(95)00366-5

Table 1. Clinical Characteristics of the Study Population

Clinical scenario	*n*	Maternal age (y)	Parity	Sampling EGA (wk)	Delivery EGA (wk)	Birth weight (g)
Term, no labor	29	28.3 ± 1.1	3.1 ± 0.4	38.2 ± 0.4	38.6 ± 0.3	3253 ± 130
Term, in labor	36	24.0 ± 1.2	2.7 ± 0.4	38.8 ± 0.4	39.2 ± 0.5	3372 ± 89
Preterm, undelivered	19	24.6 ± 1.3	1.8 ± 0.4	32.5 ± 0.9	33.9 ± 1.1*	2369 ± 240
Preterm, delivered	18	25.9 ± 1.5	2.0 ± 0.3	29.4 ± 1.1	29.8 ± 1.0	1610 ± 181
Chorioamnionitis	8	20.1 ± 1.7	1.4 ± 0.4	30.1 ± 1.4	30.1 ± 1.4	1544 ± 231

EGA = estimated gestational age.
Values are presented as mean ± standard error of the mean.
* Delivery EGA was available for 14 and birth weight for ten of the patients in this group.

women who did not deliver within 1 week of presentation were not in preterm labor. The diagnosis of clinically evident chorioamnionitis was made according to the criteria of Gibbs and Duff[14] and included the presence of maternal fever (38.2C or higher), fetal tachycardia (more than 160 beats per minute), maternal tachycardia (pulse 100 beats per minute or greater), or uterine tenderness. Most AF samples from patients with preterm contractions were obtained at presentation during clinically indicated amniocentesis. Amniotic fluid samples were collected from patients at hysterotomy in those women undergoing repeat cesarean delivery at term, whereas samples from randomly selected women in labor at term were collected at the time of clinically indicated amniotomy. An angiocatheter was inserted transcervically into the amniotic cavity under aseptic conditions, and AF was aspirated. Samples were aliquoted and stored at −70C until the time of assay. Demographic and other pertinent medical data were collected for each patient. This study was approved by the Institutional Review Board at the University of Utah.

For women at term and not in labor, specimens were collected at amniocentesis for fetal pulmonary maturity testing ($n = 7$), hysterotomy during repeat cesarean delivery ($n = 15$), or amniotomy for induction of labor ($n = 7$). All of the samples from women in labor at term were collected during amniotomy after the patient presented in labor ($n = 36$). In the 19 women who presented with preterm contractions and who remained undelivered for at least 1 week, all AF samples were collected at amniocentesis to evaluate AF for indices of infection. Delivery data was unavailable for five patients, and birth weight was unavailable for nine patients who were lost to follow-up. In the women who delivered preterm within 1 week of presentation, all of the samples were collected by amniocentesis or at hysterotomy. Samples from women with chorioamnionitis were collected at amniocentesis or after amniotomy. None of the preterm patients had received antenatal steroids before AF collection. Of women who were preterm but who did not deliver within 1 week, nine of 19 (47%) received tocolytic therapy, as did 11 of the 18 (61%) women who delivered within 1 week. The tocolytic agents used included subcutaneous terbutaline and/or intravenous magnesium sulfate.

Samples were assayed for MIP-1α using a commercially available enzyme-linked immunosorbent assay (R & D, Minneapolis, MN). For this assay, the inter-assay coefficient of variation is 7.6% and the intra-assay coefficient of variation is 3%, with a range 46.9–1500 pg/mL and a sensitivity of 2 pg/mL.

Normally distributed data were compared using unpaired *t* tests, with a significance level of $P < .05$. For comparisons of AF MIP-1α values between different groups, a Wilcoxon rank-sum test or Kruskal-Wallis test was used, with $P < .05$ deemed significant. Comparisons of AF MIP-1α with cervical dilation were performed by simple regression analysis. All statistical analyses was performed using StatView 4.01 (Abacus Concepts, Berkeley CA).

Results

Table 1 presents clinical data regarding the five groups of patients. Women at term and not in labor were older because most were undergoing repeat cesarean delivery, whereas women with preterm chorioamnionitis were younger than the other four groups of women ($P < .05$). Mean parity was also higher in the women at term and not in labor. There is no known effect of age or parity on AF cytokine concentrations. As expected, women who presented with preterm contractions and did not deliver within 1 week had a greater estimated gestational age at delivery and birth weight than did women who were in preterm labor and women with preterm chorioamnionitis.

Figure 1 depicts the concentrations of AF MIP-1α in women at term, both in labor or not in labor. There was a statistically significant elevation ($P < .001$) in the MIP-1α concentrations in the AF obtained from women at term and in labor when compared with MIP-1α AF concentrations in women at term and not in labor. Then we compared AF MIP-1α concentration with the cervi-

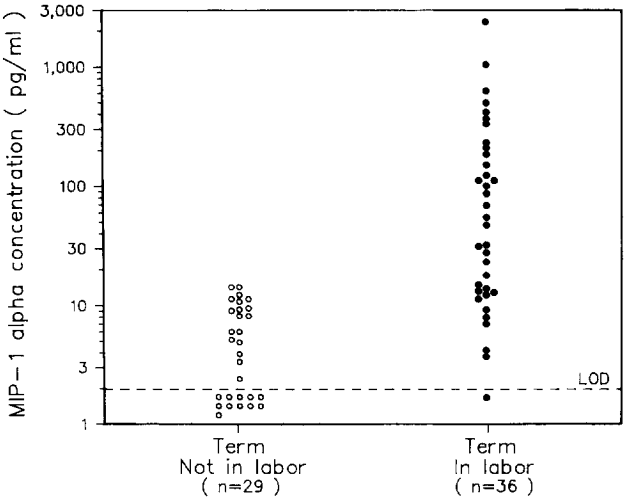

Figure 1. Concentrations of macrophage inflammatory protein-1α (MIP-1α) in amniotic fluid collected from women at term, not in labor (n = 29) and in labor (n = 44). Note that the y axis is a logarithmic scale, to discriminate more clearly the values of MIP-1α in these samples. LOD = limit of detection of the assay. Wilcoxon rank-sum test, P < .001.

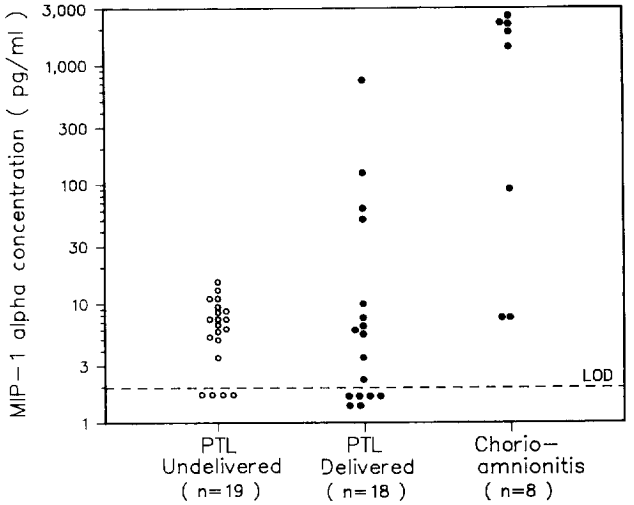

Figure 3. Concentrations of macrophage inflammatory protein-1α (MIP-1α) in amniotic fluid collected from women with preterm labor (PTL) undelivered within 1 week (n = 19), preterm labor delivered within 1 week (n = 22), and preterm chorioamnionitis (n = 8). Note that the y axis is a logarithmic scale, to discriminate more clearly the values of MIP-1α in these samples. LOD = limit of detection of the assay. Kruskal-Wallis test, P = .001.

cal dilation of the patient at the time of labor. Figure 2 depicts this relationship, and there was significant correlation (P < .001) of AF MIP-1α values and cervical dilation. As labor progressed, AF MIP-1α concentrations increased with advancing cervical dilation.

Figure 3 shows the AF MIP-1α concentrations in AF collected from women with preterm labor. The differences of AF MIP-1α among these three groups of women were statistically significant (P = .001). Women with chorioamnionitis had the highest elevations of AF MIP-1α concentrations. Next, we compared the AF MIP-1α concentration with the cervical dilation at AF collection. There was no correlation between cervical dilation and AF MIP-1α values, although the women with chorioamnionitis tended to have very high levels of AF MIP-1α early in the active phase of labor (Figure 4).

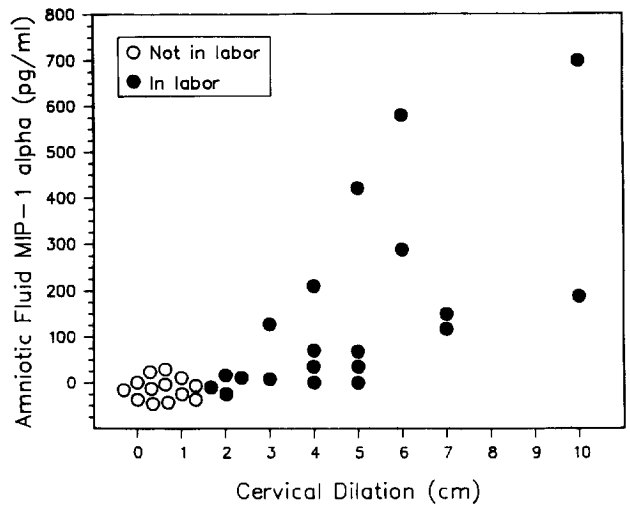

Figure 2. Comparison of amniotic fluid macrophage inflammatory protein-1α (MIP-1α) concentrations with cervical dilation in women at term. The y axis is a linear scale. There is a significant correlation of these values (r^2 = 0.479, P < .001).

Discussion

Our data show that MIP-1α concentrations are increased in the AF collected from women both in term and preterm labor, and particularly with clinically evident intrauterine infection. Moreover, in term labor, AF MIP-1α increases with advancing labor. The potential intrauterine sources of MIP-1α include maternal and fetal gestational tissues, fetal macrophages and other cells suspended in AF, and mature and immature hematopoietic-lineage fetal cells. We have found that cultured decidual cells produce MIP-1α in response to IL-1β and TNF-α[15] in a concentration-dependent fashion, indicating that maternal gestational tissues are one possible source for AF MIP-1α.

Interleukin-8 and MIP-1α are members of the chemokine, or chemoattractant cytokine, family. Chemokines are divided into two general subfamilies based upon their amino acid sequence.[13] Interleukin-8 is a member

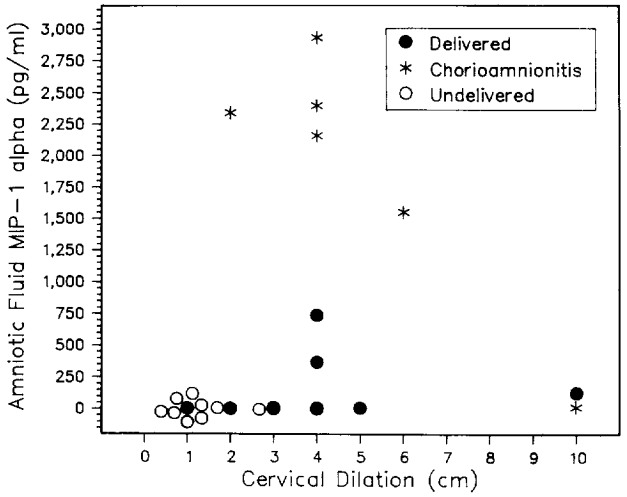

Figure 4. Comparison of amniotic fluid (AF) macrophage inflammatory protein-1α (MIP-1α) concentrations with cervical dilation in women in preterm labor. The *y* axis is a linear scale. There is no significant correlation among these values. Note that the AF MIP-1α concentrations of women with chorioamnionitis (marked by the asterisk) is elevated at early stages of labor.

of the C-X-C subfamily, whereas a prototypic chemokine of the C-C family of chemokines includes MIP-1α. The C-X-C chemokines, such as IL-8, attract neutrophils into tissues, whereas the C-C chemokines, including MIP-1α, attract monocytes, macrophages, and other immune effector cells, such as mast cells, into tissues. These chemokines activate these cells, which either degranulate or produce inflammatory mediators, such as cytokines and PGs. Among the possible roles of MIP-1α in the pathophysiology of preterm labor, establishment of a concentration gradient against which monocytes and macrophages migrate may be key.[16] Macrophage inflammatory protein-1α elaboration by gestational tissues could account for monocytic and macrophage infiltrates characteristic of chorioamnionitis, whereas elaboration of IL-8 likely accounts for neutrophil aggregation. Macrophage inflammatory protein-1α may also activate monocytes and macrophages as well as cells resident in gestational tissues. Macrophage inflammatory protein-1α stimulates the production of IL-6 and PGE_2 by murine macrophages,[17] and our preliminary data show that MIP-1α in vitro stimulates decidual cell IL-6 production, chorion cell IL-6 and PGE_2 production, and amnion cell PGE_2 production. These findings suggest that MIP-1α may act to attract and activate immune effector cells into gestational tissues, resulting in the production of other cytokines and arachidonic acid metabolites. These substances may contribute to the milieu of local and systemic factors that then stimulate uterine activity.

Our findings differ from those of Romero et al.[18] They were the first to report that concentrations of MIP-1α are elevated in the AF of women with infection-associated preterm labor, but in their study, AF MIP-1α concentrations did not rise with labor in women at term. However, all of their samples were obtained by amniocentesis, whereas our samples obtained from women in term labor were collected at amniotomy. These intriguing findings support the hypothesis that there are differences in "forebag" and "hindbag" cytokine concentrations, the so-called two-compartment theory regarding the concentrations of bioactive substances in AF.[19,20] Romero et al[19] found that PGE_2 concentrations are significantly higher in the forebag than in the hindbag, and Cox et al[20] also found that concentrations of IL-1β and IL-6 are elevated in AF collected from the forebag.

These findings prompted Cox et al[20] to speculate that elevations of cytokines and PGs in AF of women in labor are the consequence of labor rather than the cause. We agree that inflammatory cytokines are not the likely cause of labor. However, a key component of labor is the softening and effacement of the uterine cervix.[21] The collagen content of the cervix undergoes considerable remodeling in preparation for labor, with the start of labor, and as labor progresses.[22] One consequence of monocyte and macrophage activation is the release of a number of enzymes that can participate in the remodeling of tissue, including elastases and collagenases.[23,24] The tissue content of these enzymes in the cervix is positively correlated with successful labor.[25] Interleukin-8 has been shown to induce cervical ripening in rabbits[26] and guinea pigs.[27] Human and rabbit cervical cells produce IL-8[28,29] and rabbit cervical cell IL-8 production is suppressed by progesterone.[29] These investigators speculated that chemokine release by cells in the cervix may be an important physiologic event in the normal cervical maturational events preceding labor. Thus, one potential role of locally produced MIP-1α in the tissues at the forebag may be to attract and activate monocytes and macrophages in the cervical stroma, resulting in cervical softening as collagen is remodeled.

In conclusion, we found that concentrations of the chemokine MIP-1α were elevated in the AF of women in preterm and term labor and that forebag concentrations of MIP-1α increased as labor progressed. Given the postulated role of the related chemokine IL-8 in the process of cervical maturation, we suggest that MIP-1α similarly plays a key role in the physiology of normal labor as well as the pathophysiology of infection-associated preterm labor.

References

1. Romero R, Munoz H, Gomez R, et al. Does infection cause premature labor and delivery? Semin Reprod Endocrinol 1994;12: 227–39.
2. Romero R, Mazor M, Brandt F, et al. Interleukin-1α and interleukin-1β in preterm and term human parturition. Am J Reprod Immunol 1992;27:117–23.
3. Romero R, Mazor M, Sepulveda W, Avila C, Copeland D, Williams J. Tumor necrosis factor in preterm and term labor. Am J Obstet Gynecol 1992;166:1576–87.
4. Dudley DJ, Hunter C, Mitchell MD, Varner MW. Clinical value of amniotic fluid interleukin-6 determinations in the management of preterm labor. Br J Obstet Gynaecol 1994;101:592–7.
5. Greig PC, Ernest JM, Teot L, Erikson M, Talley R. Amniotic fluid interleukin-6 levels correlate with histologic chorioamnionitis and amniotic fluid cultures in patients in premature labor with intact membranes. Am J Obstet Gynecol 1993;169:1035–44.
6. Romero R, Yoon BH, Mazor M, et al. The diagnostic and prognostic value of amniotic fluid white blood cell count, glucose, interleukin-6, and Gram stain in patients with preterm labor and intact membranes. Am J Obstet Gynecol 1993;169:805–16.
7. Cherouny PH, Pankuch GA, Romero R, et al. Neutrophil attractant/activating peptide-1/interleukin-8: Association with histologic chorioamnionitis, preterm delivery, and bioactive amniotic fluid leukoattractants. Am J Obstet Gynecol 1993;169:1299–303.
8. Dudley DJ, Trautman MS, Araneo BA, Edwin SS, Mitchell MD. Decidual cell biosynthesis of interleukin-6: Regulation by inflammatory cytokines. J Clin Endocrinol Metab 1991;74:884–9.
9. Dudley DJ, Lundin-Schiller S, Edwin SS, Trautman MS, Mitchell MD. Biosynthesis of interleukin-6 by cultured human chorion laeve cells: Regulation by cytokines. J Clin Endocrinol Metab 1992;75:1081–6.
10. Dudley DJ, Trautman MS, Mitchell MD. Inflammatory mediators regulate interleukin-8 production by cultured gestational tissues: Evidence for a cytokine network at the chorio-decidual interface. J Clin Endocrinol Metab 1993;76:404–10.
11. Mitchell MD. The mechanism(s) of human parturition. J Dev Physiol 1984;6:107–18.
12. Edwin SS, LaMarche SL, Thai D, Branch DW, Mitchell MD. 5-hydroxyeicosatetraenoic acid biosynthesis by gestational tissues: Effects of inflammatory cytokines. Am J Obstet Gynecol 1993;169: 1467–71.
13. Baggiolini M, Dewald B, Moser B. Interleukin-8 and related chemotactic cytokines—CXC and CC chemokines. Adv Immunol 1994;55:97–179.
14. Gibbs RS, Duff P. Progress in pathogenesis and management of clinical intra-amniotic infection. Am J Obstet Gynecol 1991;164: 1317–26.
15. Dudley DJ, Spencer S, Edwin SS, Mitchell MD. Regulation of human decidual cell macrophage inflammatory protein-1α (MIP-1α) production by inflammatory cytokines. Am J Reprod Immunol 1995;34:231–5.
16. Neote K, DiGregorio D, Mak JY, Horuk R, Schall TJ. Molecular cloning, functional expression, and signalling characteristics of a C-C chemokine receptor. Cell 1993;72:415–25.
17. Fahey TJ, Tracey KJ, Tekamp-Olson P, et al. Macrophage inflammatory protein 1 modulates macrophage function. J Immunol 1992;148:2764–9.
18. Romero R, Gomez R, Galasso M, et al. Macrophage inflammatory protein-1α in term and preterm parturition: Effect of microbial invasion of the amniotic cavity. Am J Reprod Immunol 1994;32: 108–13.
19. Romero R, Gonzales R, Baumann P, et al. Topographic differences in amniotic fluid concentrations of prostanoids in women in spontaneous labor at term. Prostaglandins Leuko Essent Fatty Acids 1994;50:97–104.
20. Cox SM, King MR, Casey ML, MacDonald PC. Interleukin-1β, -1α, -6 and prostaglandins in vaginal/cervical fluids of pregnant women before and during labor. J Clin Endocrinol Metab 1993;77: 805–15.
21. Huzar G, Naftolin F. The myometrium and uterine cervix in normal and preterm labor. N Engl J Med 1984;311:571–81.
22. Kanayama N, Terao T. The relationship between granulocyte elastase-like activity of cervical mucus and cervical maturation. Acta Obstet Gynecol Scand 1991;70:29–34.
23. Matrisian LM. The matrix-degrading metalloproteinases. Bioessays 1992;14:455–63.
24. Rajabi M, Dean DD, Woessner JF. High levels of serum collagenase in premature labor—A potential biochemical marker. Obstet Gynecol 1987;69:179–86.
25. Granstrom LM, Ekman GE, Malmstrom A, Ulmsten U, Woessner JF. Serum collagenases levels in relation to the state of the human cervix during pregnancy and labor. Am J Obstet Gynecol 1992;67: 1284–8.
26. El Maradny E, Kanayama N, Halim A, Maehara K, Sumimoto K, Terao T. Interleukin-8 induces cervical ripening in rabbits. Am J Obstet Gynecol 1994;171:77–83.
27. Chwalisz K, Menson M, Skcholz P, Daum J, Beier HM, Hegele-Hartung C. Cervical ripening with cytokines interleukin 8, interleukin 1β, and tumor necrosis factor in guinea-pigs. Hum Reprod 1994;9:2173–81.
28. Barclay CG, Brennand JE, Kelly RW, Calder AA. Interleukin-8 production by the human cervix. Am J Obstet Gynecol 1993;169: 625–32.
29. Ito A, Imada K, Sato T, Kubo T, Matsushima K, Mori Y. Suppression of interleukin-8 production by progesterone in rabbit uterine cervix. Biochem J 1994;301:183–6.

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Received June 1, 1995.
Received in revised form August 7, 1995.
Accepted August 14, 1995.