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Interleukin-1 Type II Receptor: A Decoy Target for IL-1 That Is Regulated by IL-4

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Interleukin-1 (IL-1) interacts with cells through two types of binding molecules, IL-1 type I receptor (IL-1R I) and IL-1R II. The function of IL-1R II is unknown. In studies using monoclonal antibodies, IL-1 prolonged the in vitro survival of polymorphonuclear cells (PMN) through IL-1R I, and IL-4 antagonized the action of IL-1 by inducing expression and release of IL-1R II. Dexamethasone also induced expression and release of the IL-1R II in PMN. These results, together with the effect of antibodies to IL-1R on IL-1–induced production of cytokines in monocytes, indicate that IL-1 acts on myelomonocytic cells through IL-1R I and that IL-1R II inhibits IL-1 activity by acting as a decoy target for IL-1. The existence of multiple pathways of regulation emphasizes the need for tight control of IL-1 action.

IL-1α and IL-1β are pleiotropic cytokines that mediate a wide range of biological activities on different cell types (*1*). Two molecules in the cell membrane that bind IL-1, referred to as IL-1R I and IL-1R II, have been identified and are expressed in different amounts in cells of different lineages. IL-1R I is an 80-kD transmembrane protein, and its signaling function has been demonstrated (*2*). It represents the main form of IL-1 receptor found in fibroblasts and T lymphocytes. IL-1R II is a 68-kD molecule (*3–5*) with a relatively short (29 amino acids) cytoplasmic tail (*6*) and is predominantly expressed in B lymphocytes, monocytes, and PMN. The biological role of IL-1R II is unknown.

Human PMN have a limited life-span in vitro and in vivo. A series of cytokines and bacterial products promote survival of PMN in vitro by inhibiting the spontaneous process of programmed cell death (apoptosis) (*7, 8*). In particular, IL-1 strongly promotes survival of PMN (*7*). IL-4, a cytokine that inhibits various functions related to inflammation in myelomonocytic cells (*9*), almost completely abolishes the effect of IL-1 in promoting the survival of PMN. In searching for the mechanism underlying this phenomenon, we have examined the biological role of IL-1 receptors in PMN. Our results indicate that, although the biological activity of IL-1 in PMN is exerted through IL-1R I, IL-1R II, either membrane-bound or secreted, acts as a molecular trap for IL-1, inhibiting its activity.

Although IL-4 alone did not affect the in vitro life-span of PMN, it almost completely abolished the prolongation of survival induced by IL-1β (Fig. 1A). In results from 15 donors, the inhibitory effect of IL-4 on IL-1β–induced survival of PMN ranged from 85 to 100%. The effect of IL-4 was dose-dependent between 0.1 and 10 ng/ml (*10*) and specific for IL-1β, in that the effects of other cytokines, such as interferon-γ (IFN-γ), granulocyte-macrophage colony-stimulating factor and granulocyte colony-stimulating factor (GM-CSF and G-CSF), IL-6, and tumor necrosis factor (TNF), that prolong the survival of PMN (*7, 8*) were unaffected (Fig. 1B shows results for GM-CSF). IL-4 also partially inhibited the prolongation of survival induced by lipopolysaccharide (LPS) (Fig. 1B), an inducer of IL-1 in these cells (*11*).

PMN express IL-1R II (*4, 5*). We therefore investigated the role of IL-1 receptors in the contrasting activities of IL-1 and IL-4 on PMN life-span. PMN had high-affinity binding sites for IL-1β [217 ± 62 sites per cell, dissociation constant (K_d = 8.8 × 10^{-10} mol liter^{-1}) (Fig. 2A). By Northern (RNA) analysis, it was found that PMN expressed predominantly IL-1R II mRNA transcripts and a barely visible IL-1R I band (Fig. 2B). Cross-linking revealed the presence of a 68-kD molecule (after subtraction of the size of the ligand; Fig. 2C), a size consistent with that of IL-1R II.

Exposure of PMN to IL-4 increased expression of IL-1R II. IL-4–treated PMN showed 553 ± 105 sites per cell, whereas the K_d was unchanged (7.8 × 10^{-10} mol liter^{-1}) (Fig. 2A). IL-1β did not affect the number or affinity of binding sites on PMN, nor did it affect the action of IL-4 (*10*). Treatment of cells with IL-4 also augmented the expression of IL-1R II mRNA (Fig. 2B), and cross-linking revealed a prominent 68-kD molecule that bound IL-1β on the surface of PMN (Fig. 2C). Treatment of PMN with IL-4 also increased the steady-state amount of IL-1R I mRNA. Cross-linking with ^{125}I-labeled IL-1β of proteins released into the culture medium by PMN treated with IL-4 revealed the presence of an IL-1β–binding protein (Fig. 2D). A small amount of this cross-linked product was also released from untreated PMN (Fig. 2D). Cross-linking of the protein was inhibited by an excess of unlabeled IL-1β (Fig. 2D).

After taking into account the molecular size of the ligand, we determined that the apparent molecular size of IL-1–binding protein released by IL-4–treated PMN was ~45 kD. Soluble IL-1–binding proteins of similar molecular size have been identified in conditioned media from human mononuclear cells and from the Raji B cell line (*12*) and probably represent a soluble form of IL-1R II. Treatment of cells with IL-4

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increases the expression of IL-1R I (*13*) (Fig. 2B). To positively identify the 45-kD IL-1–binding protein released by IL-4–treated cells, we used antibodies specific for IL-1R I and IL-1R II (*6*, *14*). Monoclonal antibody (mAb) to IL-1R II (M22) blocked the cross-linking produced by ^{125}I-labeled IL-1β of the IL-1–binding protein released by IL-4–treated PMN, whereas mAbs to IL-1R I (M1 and M4) had no effect. MAbs to IL-1R I inhibit binding of IL-1 to endothelial cells and activation of those cells, which only express IL-1R I (*15*). These results identify the IL-1–binding protein

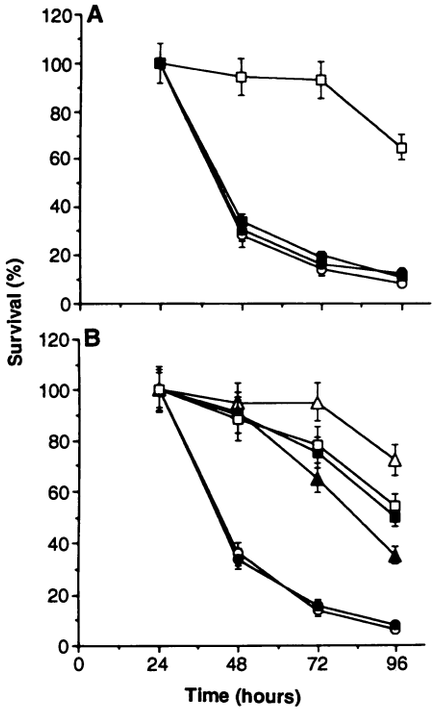

Fig. 1. Effects of IL-1β and IL-4 on survival of PMN. (**A**) Time course of PMN survival in response to IL-1β (10 ng/ml) (□) or IL-4 (10 ng/ml) (●) or both (■). After plating, cells were incubated for various periods of time (from 24 to 96 hours) in the presence or absence of cytokines. Data are expressed as percentage survival of input populations as assessed by trypan blue dye exclusion and are the mean ± SE of results obtained with cells from seven donors. (○), untreated cells. (**B**) Time course of PMN survival in response to GM-CSF (500 U/ml) with (■) or without (□) IL-4 (10 ng/ml) or to LPS (10 ng/ml) with (▲) or without (△) IL-4 (10 ng/ml). Data are the mean ± SE from five different donors. PMN were separated and cultured as described (*7*). Circulating PMN (≥98% pure) were separated by Percoll gradient centrifugation and grown in RPMI 1640 with autologous serum (10%) [10^7 cells/ml in 96-well plastic plates (0.2 ml per well) at 37°C in 5% CO_2]. The following human recombinant cytokines were used: IL-1β (10^6 U/mg), IL-4 (10^8 U/mg), and GM-CSF (10^7 U/mg). All cell counts were done in triplicate after coding of samples. (○), Untreated cells.

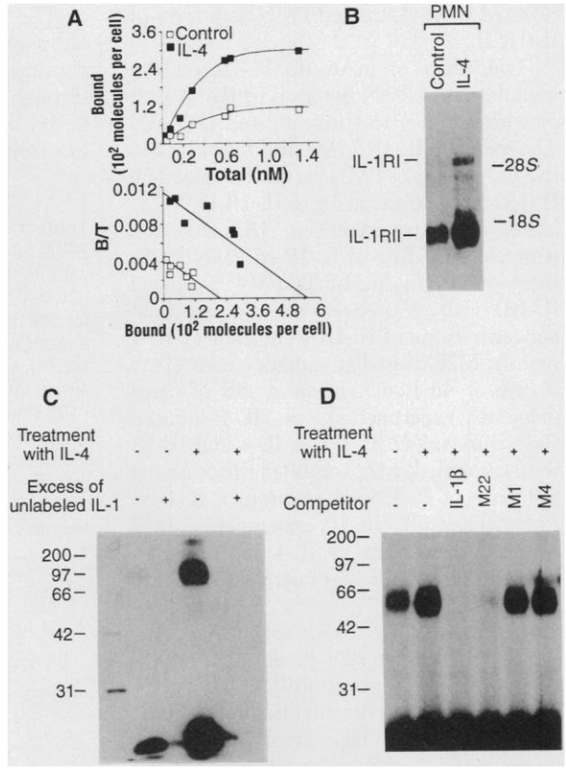

Fig. 2. Regulation of expression and release of IL-1R II by IL-4. (**A**) Saturation curve and Scatchard analysis of ^{125}I-labeled IL-1β binding to IL-4–treated PMN from one representative donor out of three tested with similar results. After treatment with IL-4 (10 ng/ml) for 18 hours at 37°C, 1 × 10^6 to 2 × 10^6 cells were incubated with various concentrations of ^{125}I-labeled IL-1β (NEN Dupont; 180 μCi/μg) in the presence or absence of a 200-fold excess of unlabeled cytokine in 0.1 ml of binding buffer [phosphate-buffered saline (PBS) with 0.1% bovine serum albumin and 0.02% sodium azide] at room temperature for 1 hour. (**B**) Northern blot analysis of transcripts encoding IL-1R I and the IL-1R II in untreated or IL-4–treated PMN. RNA extraction and analysis were done according to standard procedures (*18*). The probes were an Eco RI–Hind III fragment of 477 base pairs (bp) and an Eco RI–Sal I 750-bp fragment from, respectively, IL-1R I and IL-1R II cDNAs. Transcript sizes were ~5000 and 1600 bp for IL-1R I and IL-1R II mRNAs. (**C**) Affinity cross-linking of ^{125}I-labeled IL-1β (NEN Dupont; 130 μCi/μg) to untreated or IL-4–treated PMN. Cells were incubated with IL-4 and labeled IL-1 as described in (A). After cross-linking with disuccinimydyl suberate (DSS), cell pellet was lysed and analyzed by SDS–polyacrylamide gel electrophoresis (8% gel) under reducing conditions. Autoradiography was done for 1 to 3 days. (**D**) Affinity cross-linking of ^{125}I-labeled IL-1β to soluble IL-1R II. PMN (30 × 10^6) were cultivated with or without IL-4 (10 ng/ml, 18 hours) in RPMI 1640 without serum at 37°C. Medium was recovered and concentrated 10 times by membrane filtration (exclusion size, 10 kD). Portions (200 μl) of concentrated media were added with 1 nM ^{125}I-labeled IL-1β with or without 200 nM unlabeled cytokine or mAbs M1, M4, or M22 (10 μg/ml) and incubated at 4°C for 1 hour. After addition of 1 mM DSS at 4°C for 30 min, samples were analyzed by gel electrophoresis.

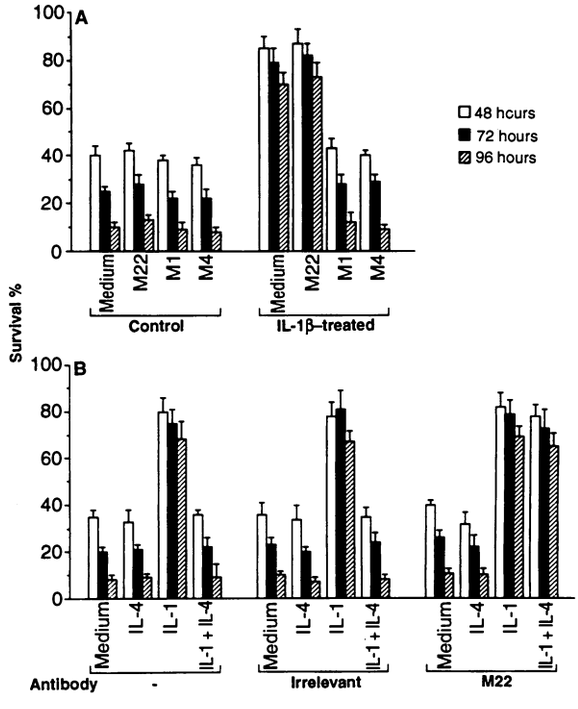

Fig. 3. Role of IL-1R I and IL-1R II in the regulation of PMN survival by IL-4 and IL-1β. (**A**) Effect of antibodies to IL-1R I (M1 and M4) and antibodies to IL-1R II (M22) on IL-1–induced PMN survival. (**B**) Effect of M22 on IL-4–mediated suppression of IL-1–induced survival of PMN. Cells were incubated with the indicated cytokines and mAbs for 48 to 72 hours. Data show the percentage of cells that survived as assessed by trypan blue dye exclusion (mean ± SE of four different experiments). In six additional experiments for (A) and seven for (B), only the 48-hour time point was examined, with similar results. An irrelevant mAb of the same class (rat immunoglobulin G2b) did not affect PMN survival. PMN were separated and cultivated as described (Fig. 1) with IL-1β (10 ng/ml) or IL-4 (10 ng/ml) or both. The mAbs were used at a final concentration of 10 μg/ml.

released by IL-4–treated PMN as a form of IL-1R II.

The effect of mAbs to IL-1R on the modulation of PMN life-span by IL-1β with or without IL-4 was investigated (Fig. 3). The mAbs to IL-1R I (M1 and M4) blocked the promotion of PMN survival induced by IL-1β. In contrast, mAb to IL-1R II (M22) had no inhibitory effect (Fig. 3A), although it blocked binding of IL-1β to PMN (90% inhibition of binding of 200 pM ^{125}I-labeled IL-1β) (*10*). Moreover, when suboptimal concentrations of IL-1β were used (1 to 2 ng/ml), M22 caused an enhancement (14.5 ± 3% at 48 hours, mean ± SE of three different experiments) of IL-1–induced PMN survival (*10*). When IL-4 and IL-1β were combined, M22 inhibited the opposing influence of IL-4 on the action of IL-1 on PMN (Fig. 3B). In 10 experiments, M22 caused a reduction in IL-4 inhibition of IL-1–induced survival ranging from 78 to 91%.

To extend these studies to another related cell type expressing predominantly IL-1R II and to a response to IL-1 other than survival, we used freshly isolated human monocytes. IL-1 induces cytokine release in monocytes (*1*). The mAb M4 blocked the induction by IL-1β of IL-6, IL-8, monocyte chemotactic protein–1 (MCP-1), and IL-1α in human monocytes (Table 1). M4 also inhibited the induction by IL-1β of augmented expression of intercellular adhesion molecule–1 (ICAM-1) in monocytes (Table 1). In contrast, M22 had no inhibitory action and augmented the activity of suboptimal concentrations (≤0.5 ng/ml) of IL-1β on cytokine production and ICAM-1 expression (Table 1).

Glucocorticoid hormones (GCs) are potent inhibitors of inflammation and immunity. GCs increase expression of IL-1R in various cell types (*5*, *16*). We therefore investigated whether GCs induced expression and release of IL-1R II in PMN. Dexamethasone (Dex) induced increased binding of IL-1β to PMN (Fig. 4A), associated with an augmented steady-state amount of IL-1R II mRNA (Fig. 4B) and an increased amount of IL-1R II at the cell surface as assessed by cross-linking (Fig. 4C). The supernatants of PMN treated with Dex contained high amounts of IL-1R II, identified by cross-linking and competition with mAbs (Fig. 4D). The effect of GCs on IL-1R II expression and release was dose-dependent, specific for anti-inflammatory steroids (testosterone, progesterone, and 17β-estradiol were ineffective), and was blocked by the specific antagonist RU486 (*10*). The induction of expression and release of a soluble form of IL-1R II in PMN may represent a pathway through which GCs exert anti-inflammatory and immunosuppressive activity.

The results presented here indicate that although myelomonocytic cells express predominantly IL-1R II, they respond to IL-1 through IL-1R I. Under these conditions, IL-1R II acts as a molecular trap for IL-1, a function enhanced by IL-4 and GCs, which augment membrane expression and release of this IL-1–binding molecule. The genome of vaccinia and cowpox viruses encodes the sequence of molecules related to IL-1R II that contribute to pathogenicity (*17*). Thus, IL-1R II appears to function as a physiolog-

Table 1. Effect of mAbs to IL-1R on monocyte activation by IL-1β. Monocytes (10^6 per milliliter in RPMI 1640 medium with 1% fetal calf serum) purified as described (*18*) were cultured for 24 hours with IL-1β (0.5 ng/ml) with or without antibodies to IL-1R I (M4) or IL-1R II (M22) (10 μg/ml). For ICAM-1 expression, monocytes were exposed to IL-1β and mAbs for 6 hours, washed, and cultured for 20 hours. Cytokines were measured in the supernatants (IL-8 or IL-6) or in cell lysate and supernatant (IL-1α). We measured IL-8 by radioimmunoassay, IL-6 as hybridoma growth factor, MCP-1 as chemotactic activity inhibited by specific antibodies, IL-1α with a commercial enzyme-linked immunosorbent assay, and ICAM-1 with the LB2 mAb and fluorescence-activated cell sorter (*19*). The results are given as the mean (±SD) of four replicates (cytokines). For ICAM-1, the median channel of fluorescence of control unstimulated cells was subtracted, and the results are absolute increases in fluorescence intensity (arbitrary units). Results are representative of six (IL-8 and IL-6), two (IL-1α), and one (MCP-1 and ICAM-1) experiments. In four of these experiments (three for IL-6 and IL-8 and one for IL-1α), different doses of IL-1β (from 0.05 to 5 ng/ml) were tested with similar results.

IL-1β	mAb	Cellular response				
		IL-6 (U/ml)	IL-8 (ng/ml)	IL-1α (pg/ml)	MCP-1 (U/ml)	ICAM-1 (increase in fluorescence)
–	–	<50	3.6 ± 0.4	34 ± 1	<1	0
–	M4	<50	12 ± 4	38 ± 4	<1	0
–	M22	<50	7 ± 4	48 ± 5	<1	0
+	–	1556 ± 1070	33 ± 8	279.3 ± 36	12	18
+	M4	<50	11 ± 0.2	51.5 ± 7.5	<1	0
+	M22	5684 ± 384	162 ± 38	1143 ± 7	27	49

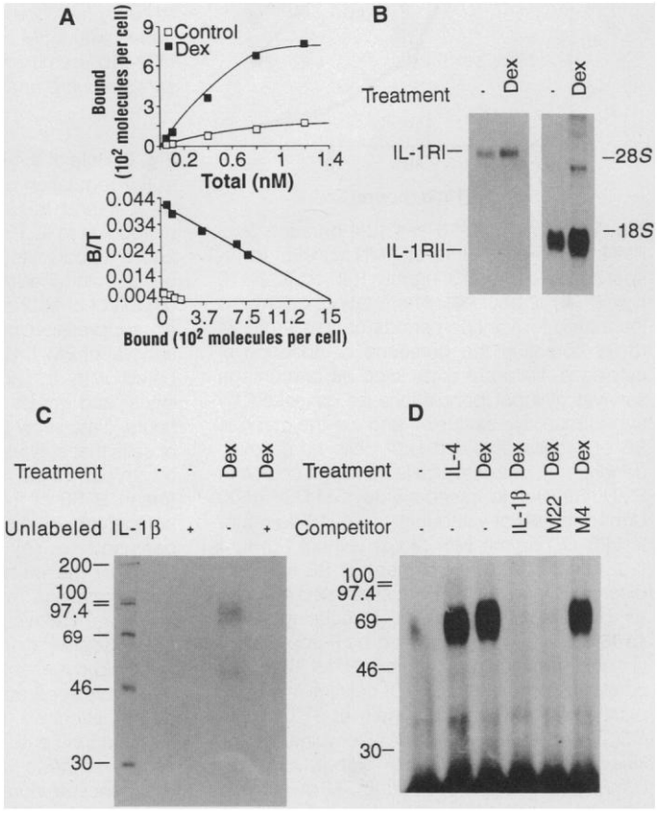

Fig. 4. IL-1R II expression and release in PMN treated with dexamethasone. (**A**) Saturation curve and Scatchard analysis of ^{125}I-labeled IL-1β (NEN Dupont; 119 μCi/μg) binding to PMN treated with Dex. In this representative donor (out of three tested with similar results) the number of receptor sites per cell were 170 ± 11.9 and 1500 ± 200 for untreated and Dex-treated PMN, respectively. Dissociation constants were 9.8 ± 0.64 × 10^{-10} and 9.9 ± 0.3 × 10^{-10} mol liter^{-1}, respectively. (**B**) Northern blot analysis of IL-1R. (**C**) Surface affinity cross-linking to ^{125}I-labeled IL-1β (119 μCi/μg). (**D**) Affinity cross-linking of ^{125}I-labeled IL-1β to soluble IL-1R II released by PMN pretreated with Dex (10^{-7} M). Experimental conditions were as described in Fig. 2. Dex was added for 12 hours (A, C, and D) or 4 hours (B).

ical pathway in the regulation of IL-1 activity in myelomonocytic cells that can be subverted by pathogens and may provide a target for pharmacological intervention.

The existence of multiple pathways of regulation, including a polypeptide receptor antagonist (*1*) and the decoy target for IL-1 binding, emphasizes the need for tight regulation of IL-1 activity. We suggest that the term "receptor" may be an inappropriate designation, at least until evidence is obtained that IL-1R II binding protein is indeed a signaling receptor, and propose to refer to this acceptor molecule as a "decoy receptor."

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Right Hemisphere Dominance for the Production of Facial Expression in Monkeys

Marc D. Hauser

In humans, the left side of the face (right hemisphere of the brain) is dominant in emotional expression. In rhesus monkeys, the left side of the face begins to display facial expression earlier than the right side and is more expressive. Humans perceive rhesus chimeras created by pairing the left half of the face with its mirror-reversed duplicate as more expressive than chimeras created by right-right pairings. That the right hemisphere determines facial expression, and the left hemisphere processes species-typical vocal signals, suggests that human and nonhuman primates exhibit the same pattern of brain asymmetry for communication.

In humans, neuroanatomical differences between the right and left hemispheres of the brain are associated with differences in the production and perception of behavior (*1–3*). Although nonhuman animals show both neuroanatomical and behavioral asymmetries (*2*, *4–6*), humans show stronger and more varied asymmetries (*1*, *2*). In general, the left hemisphere dominates in linguistic function and manual control, whereas the right hemisphere dominates in spatial reasoning, emotional perception, and face recognition.

Studies of some nonhuman primate populations have provided evidence that hand preference is nonrandom during both unimanual and bimanual motor tasks (*4*). Evidence of lateralization for auditory and visual perception is, however, more ambiguous. In the Japanese macaque, the left hemisphere is dominant with regard to the perception of species-typical vocal signals (*7*, *8*). Thus, both human and nonhuman primates exhibit left hemisphere dominance for referentially relevant stimuli. An assumption underlying this claim, however, is that the vocalizations used in these experiments provide referentially salient information rather than affective information. The relative contribution of referential and affective information to signal structure is currently unclear (*9*, *10*). Experiments on face recognition in monkeys and apes sometimes show a strong right hemisphere bias (*11*, *12*) but may also show more symmetric contributions of left and right hemispheres (*13*). To my knowledge, no study has examined hemispheric asymmetries in the production of vocal signals in nonhuman primates (*14*). However, a study demonstrated that, when split brain rhesus macaques were presented with videotaped sequences of human and nonhuman primate scenes to the right hemisphere, they showed significantly more facial expressions, both submissive and aggressive, than when sequences were presented to the left hemisphere (*15*).

Video records of free-ranging rhesus monkeys living on the island of Cayo Santiago, Puerto Rico, were analyzed to assess hemispheric asymmetries in the production of facial expressions (*16*). All data were collected from individually recognizable animals from one social group. Most individuals from the sample were genetically unrelated males. Frame-by-frame (one frame = 33 ms) analyses (*16*) were carried out for all expressions for which the subject's entire face was oriented toward the video camera.

Four facial expressions were evaluated, representing three different emotional states. The fear grimace (Fig. 1), which is produced by retracting the lips, is given by a subordinate individual being attacked or intimidated by a higher ranking group member. The copulation grimace, which is also produced by retracting the lips but which is not held in position as long as the fear grimace, is given by adult males during copulation. The open mouth threat, which is produced by slightly protruding the lips and placing them into an O-shaped configuration, is given by a dominant individual attacking or intimidating a subordinate. The ear flap threat, which is produced by retracting the ears back against the head, is also given by a dominant individual attacking or intimidating a subordinate.

I calculated asymmetries in the temporal emergence of an expression by comparing the onset of facial movement on the

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