Effects of IL-1 receptor accessory protein on IL-1 binding

H. Wesche[1], K. Resch, M.U. Martin*

Institut für Molekularpharmakologie, Medizinische Hochschule Hannover, D-30625 Hannover, Germany

Received 23 March 1998

Abstract Interleukin-1 (IL-1) is an important pro-inflammatory cytokine that exerts its diverse biological functions by binding to a receptor complex consisting of two transmembrane proteins, type I IL-1 receptor (IL-1RI) and IL-1 receptor accessory protein (IL-1RAcP). Both receptor chains are indispensable for IL-1 signaling, but only IL-1RI is able to bind the cytokine. The effects of IL-1RAcP on IL-1 binding are unclear. This study shows that although expression of IL-1RAcP does not alter the equilibrium dissociation constant of IL-1, it has an effect on the non-equilibrium binding modus, most likely due to changes in on/off rates. This defines an additional function of IL-1RAcP: it stabilizes the active IL-1 receptor complex.

Key words: Interleukin-1 receptor; IL-1 receptor accessory protein; Interleukin-1 binding

1. Introduction

The pro-inflammatory cytokines interleukin-1α (IL-1α) and IL-1β, hereafter called IL-1, bind to two types of specific plasma membrane receptors of which only the type I IL-1 receptor (IL-1RI) transduces signals [1] while the type II IL-1 receptor (IL-1RII) functions as decoy receptor without participating in IL-1 signaling [2]. Signal transduction by IL-1RI is only possible in concert with the IL-1 receptor accessory protein (IL-1RAcP) [3,4] which itself does not recognize the ligand [5] but interacts with the ligated IL-1RI on the outside of the plasma membrane. This results in the association of the cytoplasmic domains of IL-1RI and IL-1RAcP which are both required for IL-1 signal transduction [6–11]. Subsequently the adapter protein MyD88 associates with the cytoplasmic domains of IL-1RI and IL-1RAcP allowing the IL-1 receptor associated protein kinase (IRAK) to translocate into this complex [11,12]. Here IRAK becomes activated, presumably by autophosphorylation, allowing subsequent signaling events to take place [13,14].

Although the structure of IL-1 bound to IL-1RI has been unraveled on an atomic scale recently [15,16], it remains unclear how IL-1RAcP recognizes the ligated receptor and which immediate consequences this has. A five-fold increase in IL-1 affinity was reported in CHO cells stably transfected with IL-1RI and IL-1RAcP [5], whereas no significant differences in IL-1 affinities were observed in murine EL-4 cells which either expressed or lacked IL-1RAcP [3,4]. This discrepancy may be due to the use of different cellular models, cross-species problems, and to the relative insensitivity of Scatchard plot analysis in cells expressing low numbers of IL-1 receptors.

As we could not observe a significant influence of IL-1RAcP on the affinity of IL-1 binding, we investigated the possibility of IL-1RAcP affecting the binding of IL-1 in a different manner. Here we report (1) that in the presence of IL-1RAcP the access of antibodies to the ligand binding site in IL-1RI is obscured and (2) that the dissociation kinetics of IL-1 from the IL-1RI are altered. These data suggest that IL-1RAcP sterically protects IL-1 in the ligand binding site of IL-1RI. It can be hypothesized that by forming a 'cage' for IL-1 IL-1RAcP slows the dissociation kinetic of IL-1 from the receptor complex.

2. Materials and methods

2.1. Cell culture

The murine T cell lines EL-4 6.1 (kind gift of R. McDonald, Lausanne, Switzerland) and EL-4 D6/76 (kind gift of W. Falk, Regenburg, Germany) were maintained at 37°C, 5% CO_2 in RPMI 1640 supplemented with 5% (v/v) fetal calf serum, 1 mmol/l pyruvate, non-essential amino acids (minimum essential medium) and 2 mmol/l L-glutamine (Gibco). The cells were passaged every 2–3 days to maintain a cell density of 10^5–10^6 cells/ml.

2.2. Scatchard analysis

IL-1α (10^8 units/mg, kindly provided by John Sims, Immunex, Seattle, WA, USA) was labeled with ^{125}I using the chloramine T method as described elsewhere [17]. To determine the affinity of IL-1 receptor complexes 5×10^6 cells in 150 μl medium additionally supplemented with 0.1% (w/v) sodium azide were pre-incubated for 30 min on ice with or without 100-fold molar excess of unlabeled IL-1. To each vial [^{125}I]IL-1α was added in a concentration range between 5 and 3840 pmol/l. After 3 h of incubation on ice the cell suspension was layered on top of a cushion consisting of silicon oil (AR20/AR200 1:1, Wacker Chemikalien, Munich, Germany). The tubes were centrifuged (2 min, 5000 rpm, 4°C), decapitated and both parts (upper compartment: free IL-1; lower compartment: cells with bound IL-1) were counted using a gamma counter.

The affinity of the receptor complexes and the number of binding sites were calculated using the equation

$$\frac{\nu}{(\text{ligand}_{\text{free}})} = \frac{n}{K_{\text{diss}}} - \frac{\nu}{K_{\text{diss}}}$$

with $\nu = c(\text{ligand}_{\text{bound}})/c(\text{cells})$ and n = number of binding sites [18]. By plotting $n/c(\text{ligand}_{\text{free}})$ versus n the dissociation constant K_{diss} is obtained by the negative slope and the number of binding sites by the abscissa intercept.

2.3. Competition studies with unlabeled IL-1 and IL-1RI antibodies (FACS analysis)

10^6 cells were washed twice in PBS/1% (v/v) FCS/0.1% (w/v) NaN_3 and pre-incubated in 200 μl of PBS with 280 ng/ml IL-1α or IL-1β (rhIL-1α: $>10^8$ units/mg, Immunex; rhIL-1β, $5–10\times10^8$ units/mg, Dompé, L'Aquila, Italy) for 10 min at room temperature. The cells were washed again and resuspended in 20 μl Endobulin (Immuno, Heidelberg, Germany), 50 μl PBS/FCS/NaN_3 and 10 μl of first antibody (mIL-1RI antibodies 35F5 or 12A6, Pharmingen, Hamburg, Germany, final concentration 0–5 μg/ml). After incubation for 15 min at room temperature followed by 20 min on ice the cells were washed twice, resuspended in 100 μl DTAF-conjugated anti-rat IgG

*Corresponding author. Fax: (49) (511) 532 4081.
E-mail: Martin.Michael@MH-Hannover.de

[1]Present address: Tularik Inc., 2 Corporate Drive, South San Francisco, CA 94080, USA.

PII S0014-5793(98)00468-2

antibody (Dianova, Hamburg, Germany, 1:200 in PBS/FCS/NaN_3), incubated on ice for 30 min, and finally washed twice with PBS/FCS/NaN_3. The fluorescence intensity of the samples was measured using a fluorescence activated cell scanner (FACScan, Becton Dickinson, Heidelberg, Germany).

2.4. Competition studies with radiolabeled IL-1 and IL-1RI antibodies

2.5×10^6 cells in 150 μl medium additionally supplemented with 0.1% (w/v) sodium azide were incubated on ice with 5 ng/ml [^{125}I]IL-1α for 1.5 h. Cells were washed twice by centrifugation with medium/NaN_3 and incubated with the neutralizing mIL-1RI antibody 35F5 (Pharmingen, final concentrations 0–22 nmol/l) for 1.5 h on ice. The cell suspensions were centrifuged through silicone oil as described in Section 2.2 and the IL-1 bound was measured by gamma counting.

2.5. Competition studies with radiolabeled and unlabeled IL-1

5×10^6cells were incubated with 10 ng/ml [^{125}I]IL-1α for 30 min on ice. Then different amounts of unlabeled IL-1α or unlabeled IL-1β (final concentrations 0–188 nmol/l) were added and the samples were incubated for an additional 60 min. Separation of free and bound IL-1 and detection was performed as described above.

3. Results

3.1. IL-1RAcP has no effect on IL-1 affinity to IL-1RI

The effect of IL-1RAcP expression on IL-1 affinity was analyzed in two sublines of the murine T cell line EL-4. EL-4 6.1 is a frequently employed IL-1-responsive cell line expressing both IL-1RI and IL-1RAcP [19] whereas EL-4 D6/76 was initially identified through its inability to respond to IL-1 [20]. This non-responsiveness is due to the lack of IL-1RAcP expression and can be restored by transfection with expression plasmids encoding this protein [3,4].

Both cell lines are known to express relatively high numbers of IL-1RI, whereas IL-1RII is not detectable at the protein level and only in trace amounts via RT-PCR [19].

In order to compare the IL-1 affinity of these two sublines they were incubated with different concentrations of [^{125}I]IL-1α until equilibrium was reached (3 h on ice). The number of IL-1 binding sites and the binding affinity was determined via Scatchard plot analysis (Fig. 1). Both cell lines expressed similar numbers of IL-1 binding sites (1167 for EL-4 6.1 and 1691 for EL-4 D6/76). The dissociation constant for IL-1 was almost identical in these two sublines (1.6 nmol/l for EL-4 6.1 and 1.8 nmol/l for EL-4 D6/76). In equilibrium the two cell lines showed no difference in IL-1 affinity, although EL-4 D6/76 does not express IL-1RAcP. Thus, in the EL-4 system IL-1RAcP expression had no effect on the affinity of IL-1 binding by IL-1RI.

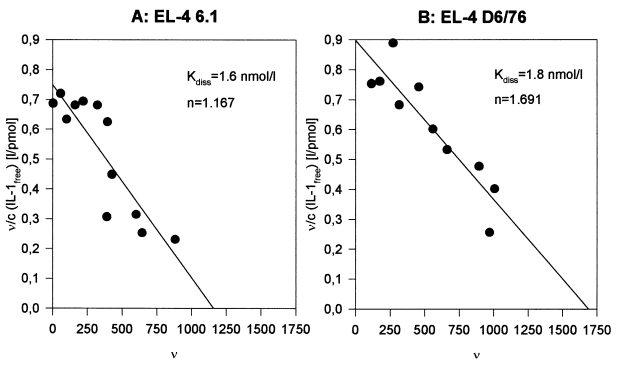

Fig. 1. Effect of IL-1RAcP expression on IL-1 affinity (Scatchard analysis). EL-4 6.1 (IL-1RAcP-positive, panel A) and EL-4 D6/76 cells (IL-1RAcP-negative, panel B) were incubated with different concentrations of [^{125}I]IL-1α. Non-specific binding was determined in the presence of a 100-fold excess of unlabeled IL-1. The IL-1 bound was determined using a gamma counter. Dissociation constants (K_{diss}) and numbers of binding places (n) were calculated by Scatchard analysis.

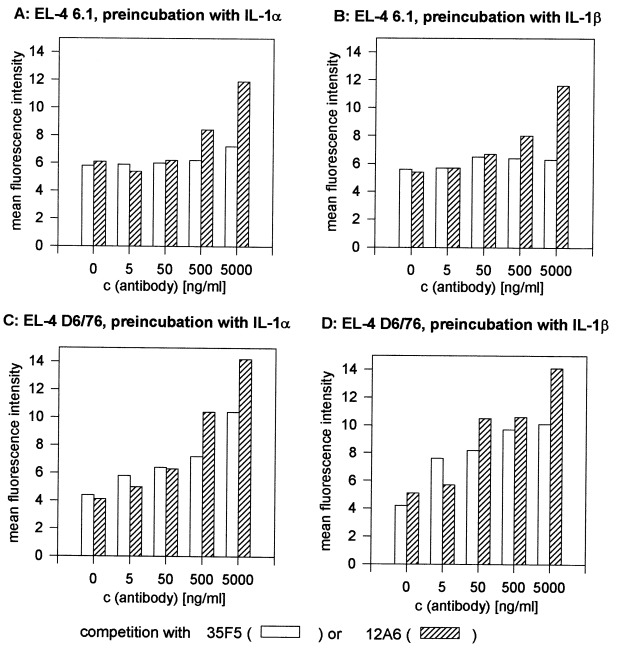

Fig. 2. Competition of IL-1 and antibody binding (FACS analysis). EL-4 6.1 (A and B) and EL-4 D6/76 cells (C and D) were pre-incubated with 280 ng/ml IL-1α (A and C) or IL-1β (B and D). For competition of IL-1 binding the cells were incubated either with the neutralizing IL-1RI antibody 35F5 or with the non-neutralizing IL-1RI antibody 12A6 (control). Binding of the IL-1RI antibodies was detected with DTAF-labeled secondary antibody.

3.2. IL-1RAcP protects bound IL-1 from competition with neutralizing antibodies

Competition studies were utilized to study the influence of IL-1RAcP on IL-1 binding in more detail. EL-4 6.1 and EL-4 D6/76 were pre-incubated with IL-1α or IL-1β and after removal of unbound IL-1 incubated with different concentrations of either a neutralizing monoclonal antibody (mAb) against IL-1RI (35F5) or a non-neutralizing mAb (12A6) for a short period of time (competition). While 35F5 is competing with IL-1 for the IL-1RI binding site, binding of 12A6 is independent of IL-1 and was used as a control to show that the level of surface expression of IL-1RI was not altered during the experiment.

The competition was quantified either by measuring the amount of bound mAb by FACS analysis (Fig. 2) or by determining the amount of bound, radiolabeled IL-1 after competition with the mAbs in binding assays (Fig. 3).

Under these experimental conditions (non-equilibrium) binding of the neutralizing mAb 35F5 was influenced by IL-1RAcP expression: when EL-4 6.1 (IL-1RAcP-positive) were pre-incubated with IL-1α or IL-1β, the antibody could not be detected on the cell surface, whereas the non-neutralizing control antibody 12A6 bound freely in a concentration-dependent manner (Fig. 2A,B). In this experimental setting the IL-1RAcP-negative cell line EL-4 D6/76 behaved differently. Both antibodies, the neutralizing and the non-neutralizing one, bound concentration-dependently (Fig. 2C,D), demon-

strating that competition of the antibodies with IL-1 for the ligand binding site in IL-1RI was only possible in the absence of IL-1RAcP.

Determination of bound IL-1 yielded the same result. In EL-4 6.1 the amount of bound IL-1 was not influenced by incubation with 35F5, whereas in EL-4D6/76 mAb 35F5 and IL-1 were competing: the higher the concentration of 35F5 in the competition experiment, the less IL-1 was found on the cell surface (Fig. 3). Thus, although IL-1RAcP did not influence the affinity of the IL-1 receptor complex in equilibrium, it altered the 'accessibility' of the bound cytokine.

3.3. IL-1RAcP prevents competition of free IL-1 with IL-1 bound to IL-1RI

The stability of IL-1 binding in the presence or absence of IL-1RAcP was further analyzed by studying the exchange of bound ligand. EL-4 6.1 and EL-4 D6/76 were pre-incubated with radiolabeled IL-1α for 30 min followed by 60 min incubation with different concentrations of unlabeled IL-1α or unlabeled IL-1β.

Only a relatively minor fraction of the radiolabeled cytokine was exchanged against unlabeled IL-1 in the IL-1RAcP positive cell line EL-4 6.1, while a much bigger fraction of the [^{125}I]IL-1α was displaced by unlabeled IL-1 in EL-4 D6/76 at even lower concentrations of competitor. Similar results were obtained for both IL-1α (data not shown) or IL-1β as competitor (Fig. 4).

4. Discussion

IL-1RAcP immediately attracted the attention of the scientific community after its initial description in 1995 [5], and it has become quite clear that IL-1RAcP is required as a co-receptor for IL-1 signaling. Although IL-1RAcP is a member of the growing IL-1 receptor family and shows significant homology to IL-1RI and IL-1RII, it is unable to bind IL-1 itself. IL-1RAcP can be detected in the receptor complex by chemical crosslinking [5] or co-immunoprecipitation [9] only

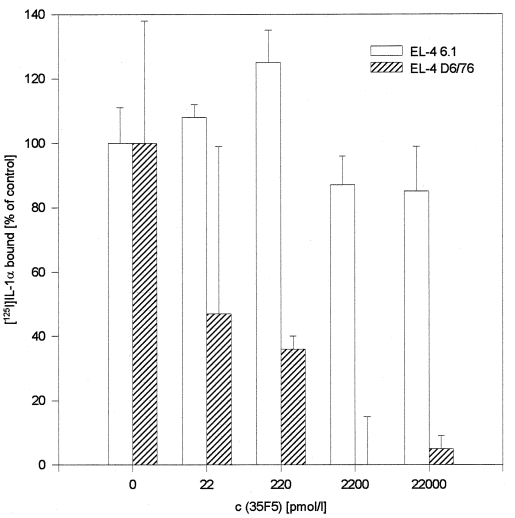

Fig. 3. Competition of [^{125}I]IL-1α and antibody binding. EL-4 6.1 and EL-4 D6/76 cells were pre-incubated with 5 ng/ml [^{125}I]IL-1α. For competition of IL-1 binding the cells were incubated with the neutralizing IL-1RI antibody 35F5. The IL-1 bound after the competition was measured using a gamma counter.

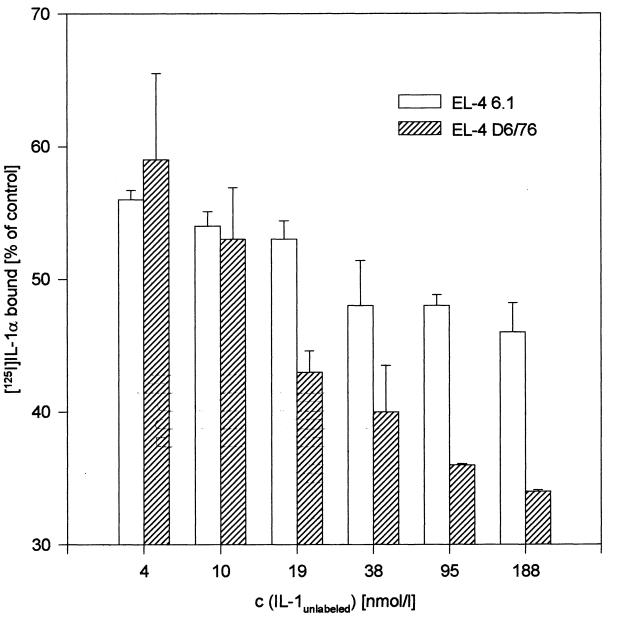

Fig. 4. Competition of ^{125}I-labeled IL-1α and unlabeled IL-1β. EL-4 6.1 and EL-4 D6/76 cells were pre-incubated with 10 ng/ml [^{125}I]IL-1α. For competition of IL-1 binding the cells were incubated with unlabeled IL-1β for 60 min on ice. The amount of bound [^{125}I]IL-1α was measured by gamma counting.

upon binding of IL-1α or IL-1β, but not IL-1Ra to IL-1RI. This suggests that IL-1RI and IL-1RAcP are not constitutively associated but form a heterodimeric complex in the presence of the biologically active IL-1 molecules. This ligand-mediated heterodimerization is a minimal working model and nothing is currently known of the stoichiometry of this complex. Interestingly, the intracellular domains do not seem to be required for the ligand-mediated association and heterodimerization, as a truncated form of IL-1RAcP can be co-precipitated with IL-1RI in the presence of IL-1 [9]. However, it is well established that the cytoplasmic domains of both IL-1RI and IL-1RAcP are indispensable for initiation of IL-1 signal transduction [6,9,11].

Recently, the structures of IL-1β and IL-1Ra bound to soluble IL-1RI were resolved by X-ray crystallography, allowing further insights into the interaction of ligand and receptor [15,16]. These data suggest that IL-1β and IL-1Ra are recognized and bound by the two N-terminally situated immunoglobulin-like domains of IL-1RI which seem to be rather rigidly connected. Upon binding by these domains only IL-1β is able to interact (via charged residues) with the third Ig-like domain leading to a conformational change, while IL-1Ra is unable to cause such a change in the overall structure of the ligated receptor. As no co-crystallization of ligated IL-1RI and IL-1RAcP has been reported, it remains elusive whether IL-1RAcP recognizes epitopes of both receptor and ligand which have been exposed or created after the conformational change or whether only bound IL-1 is seen. One hint comes from the observation that IL-1Ra can be changed into an agonist by exchanging only one amino acid (residue Lys^{145} in IL-1Ra into Asp in IL-1β) [21]. As this Asp^{145} does not seem to be involved in receptor interaction, it was speculated that it may be involved in interaction with IL-1RAcP [16].

This form of molecular interaction between ligated IL-1RI and co-receptor points to at least two possible functions for

IL-1RAcP in the process of IL-1 binding. Firstly it could enhance the affinity of the receptor complex, and secondly it could affect ligand receptor interaction by stabilizing the complex.

The aim of this study was to characterize the function of IL-1RAcP in IL-1 binding to IL-1RI in EL-4 cells. In contrast to the original description in which an increase in IL-1 affinity to its receptor by IL-1RAcP was reported [5], no evidence for differences in IL-1 affinities was seen in EL-4 cells in the absence or presence of IL-1RAcP. In stably transfected CHO cells expressing 7×10^5 receptors, IL-1RI is a binding site with a K_D of 1.2 nM, whereas upon co-expression of IL-1RI and IL-1RAcP this affinity increases about five-fold to 0.25 nM and thus constitutes the high affinity IL-1 binding site. This model is similar to the situation found in other cytokine receptor families, where the ligand binding site has a rather moderate or low affinity for the ligand which is changed into a high affinity site by a co-receptor chain which itself does not necessarily have to bind the ligand, e.g. in the IL-2 system. In EL-4 cells a significant change in K_D values in EL-4 cells was achieved neither by transfecting the murine EL-4 cell line D6/76 with IL-1RAcP [3,4] nor by comparing the affinities of IL-1RAcP-positive and -negative EL-4 cell lines (Fig. 1). In these Scatchard plot analyses only one type of binding site was measured and no evidence was found for high and low affinity binding sites for IL-1 on EL-4 cells in the absence or presence of IL-1RAcP. Therefore it must be concluded that in EL-4 cells IL-1RAcP does not contribute significantly to the equilibrium binding of IL-1 to IL-1RI and does not affect binding affinities.

As we hypothesized that IL-1RAcP may have a different function in the receptor complex we investigated the second conceivable function of IL-1RAcP: the stabilizing function. The interpretation of the three-dimensional structure suggests that by associating with the ligated receptor IL-1RAcP may cover IL-1 in its binding pocket and thus stabilize the interaction between IL-1RI and IL-1 by locking IL-1 in this microenvironment. This would deny a normal off dissociation of the ligand from the receptor and would imply that the ligand binding site is differently accessible in absence or presence of IL-1RAcP. Here we demonstrated that this is indeed the case. Firstly, we employed the neutralizing anti-murine IL-1RI mAb 35F5 which binds to the ligand binding site in IL-1RI and competes with IL-1 for the binding site. In non-equilibrium studies release of pre-bound IL-1α or IL-1β by the mAb was only possible in the EL-4 D6/76 cells, which lack IL-1RAcP, but not in EL-4 6.1 cells, which posses IL-1RAcP. Thus the accessibility of the neutralizing mAb to its epitope was denied by IL-1RAcP, most likely by steric hindrance. This was true not only for competition with the neutralizing antibody but also with IL-1α or IL-1β. Again a much higher exchange of pre-bound (radiolabeled) IL-1 with free IL-1 was possible in the cells lacking IL-1RAcP. This effect can only be explained by a protective function of IL-1RAcP. Both set of results prove that the access to the ligand binding site is obscured by IL-1RAcP and that dissociation of pre-bound IL-1 out of the receptor complex is affected negatively by IL-1RAcP which possibly creates a micro-environment favoring re-association of the ligand to the receptor binding site rather than dissociation out of the complex. This effect would prolong off rates and change the non-equilibrium binding of IL-1 to its receptor, rather than increasing the equilibrium affinity. Thus we propose that IL-1RAcP functions as a stabilizer of the IL-1 receptor signaling complex in EL-4 cells. These two molecular mechanisms are not mutually exclusive and it is possible that in other cellular systems IL-1RAcP functions in both directions by moderately increasing the affinity of the receptor for IL-1 and by stabilizing the IL-1 receptor signaling complex.

Acknowledgements: We thank Diana Boraschi, L'Aquila, Italy, and Werner Falk, Regensburg, Germany, for support and many helpful discussions and John Sims, Immunex, Seattle, WA, USA for providing us with rhIL-1α.

References

[1] Sims, J.E., Gayle, M.A., Slack, J.L., Anderson, M.R., Bird, T.A., Giri, J.G., Colotta, F., Re, F., Mantovani, A., Shanebeck, K., Grabstein, K.H. and Dower, S.K. (1993) Proc. Natl. Acad. Sci. USA 90, 6155–6159.
[2] Colotta, F., Re, F., Muzio, M., Bertini, R., Polentarutti, N., Sironi, M., Giri, J.G., Dower, S.K., Sims, J.E. and Mantovani, A. (1993) Science 261, 472–475.
[3] Wesche, H., Korherr, C., Kracht, K., Falk, W., Resch, K. and Martin, M.U. (1997) J. Biol. Chem. 272, 7727–7731.
[4] Korherr, C., Hofmeister, R., Wesche, H. and Falk, W. (1997) Eur. J. Immunol. 27, 262–267.
[5] Greenfeder, S.A., Nunes, P., Kwee, L., Labow, M., Chizzonite, R.A. and Ju, G. (1995) J. Biol. Chem. 270, 13757–13765.
[6] Heguy, A., Baldari, C.T., Macchia, G., Telford, J.L. and Melli, M. (1992) J. Biol. Chem. 267, 2605–2609.
[7] Kuno, K., Okamoto, S.-i., Hirose, K., Murakami, S. and Matsushima, K. (1993) J. Biol. Chem. 268, 13510–13518.
[8] Leung, K., Betts, J.C., Xu, L. and Nabel, G.J. (1994) J. Biol. Chem. 269, 1579–1582.
[9] Huang, J., Xao, G., Li, S. and Cao, Z. (1997) Proc. Natl. Acad. Sci. USA 94, 12829–12832.
[10] Korherr, C., Hofmeister, R. and Falk, W. (1997) Immunobiology 197, 206 (abstract F.11).
[11] Wesche, H., Henzel, W.J., Shillinglaw, W., Li, S. and Cao, Z. (1997) Immunity 7, 837–847.
[12] Muzio, M., Ni, J., Feng, P. and Dixit, V.M. (1997) Science 278, 1612–1615.
[13] Cao, Z., Henzel, W.J. and Gao, X. (1996) Science 271, 1128–1131.
[14] Cao, Z., Xiong, J., Takeuchi, M., Kurama, T. and Goeddel, D.V. (1996) Nature 383, 443–446.
[15] Vigers, G.P.A., Anderson, L.J., Caffes, P. and Brandhuber, B.J. (1997) Nature 386, 190–194.
[16] Schreuder, H., Tardif, C., Trump-Kallmeyer, S., Soffientini, A., Sarubbi, E., Akeson, A., Bowlin, T., Yanofsky, S. and Barrett, R.W. (1997) Nature 386, 194–200.
[17] Greenwood, F.C., Hunter, W.M. and Glover, J.S. (1963) Biochem. J. 89, 114.
[18] Cantor, C.R. and Schimmel, P.R. (1980) Biophysical Chemistry, W.H. Freeman and Company, New York.
[19] Wesche, H., Neumann, D., Resch, K. and Martin, M.U. (1996) FEBS Lett. 391, 104–108.
[20] von Hoegen, I., Falk, W., Kojouharoff, G. and Krammer, P.H. (1989) Eur. J. Immunol. 19, 329–334.
[21] Ju, G., Labriola-Tompkins, E., Campen, C.A., Benjamin, W.R., Karas, J., Plocinski, J., Bionbi, D., Kaffka, K.L., Kilian, P.L., Eisenberg, S.P. and Evans, R.J. (1991) Proc. Natl. Acad. Sci. USA 88, 2658–2662.