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A Monoclonal Antibody Directed Against the Murine Macrophage Surface Molecule F4/80 Modulates Natural Immune Response to Listeria monocytogenes

Holger Warschkau and Albrecht F. Kiderlen

Whole spleen cell cultures from SCID mice release high levels of IFN-γ when exposed to heat-killed Listeria monocytogenes (HKL). This microbe-induced and T cell-independent response depends on both macrophages (MΦ) and NK cells; HKL-stimulated MΦ release TNF-α and IL-12, which together activate NK cells for IFN-γ release. We show here that this cytokine-mediated activation cascade can be modulated by a mAb against the MΦ surface glycoprotein F4/80. HKL-induced IL-12, TNF-α, and IFN-γ in SCID whole spleen cell cultures was inhibited by coincubation with anti-F4/80 mAb whereas IL-1 and IL-10 were enhanced. Both effects were apparent at mRNA and protein release levels. Whereas inhibitory activities were F4/80 Ag specific, stimulatory effects were Fe dependent and nonspecific. Furthermore, cytokine inhibition by anti-F4/80 was only apparent when MΦ and NK cells were present simultaneously and in close vicinity, indicating that direct cell-to-cell contact is a prerequisite. These data suggest a novel pathway for microbe-induced MΦ/NK cell interaction involving direct cell-to-cell signaling and give the first evidence for a functional role of the MΦ surface glycoprotein F4/80. The Journal of Immunology, 1999, 163: 3409–3416.

Non-specific or natural immune responses form a first line of defense against invading pathogens and have decisive regulatory functions for subsequent Ag-specific T cell-dependent immune reactions. IFN-γ plays a central role in regulating both stages of defense and is the crucial cytokine for initiating the effector phase of cell-mediated immunity by activating MΦ for enhanced microbicidal activity (1, 2). Murine listeriosis is a widely used model for studying the mechanisms of natural cellular immune response to microorganisms. During infection with the Gram-positive facultative intracellular bacterium Listeria monocytogenes, IFN-γ production is necessary for control of bacterial growth both in immunocompetent and in SCID mice, which lack functional T and B cells (3, 4). In SCID mice or SCID whole spleen cell (WSC) cultures, L. monocytogenes can induce IFN-γ release only in the presence of both MΦ and NK cells (5). As activation pathway, a sequence of steps has been described in which Listeria first stimulate MΦ to release IL-12 and TNF-α, which in combination activate NK cells for IFN-γ release (6–8). In a positive feedback circle, IFN-γ may further activate MΦ for enhanced cytokine-synthesis and release of antimicrobial and cytotoxic metabolites such as reactive oxygen and nitrogen intermediates (9, 10).

In vitro experiments performed in our laboratory as well as by others (6) revealed that the level of IFN-γ release not only depends on the relative numbers of microorganisms but also on the culture system itself. Direct coincubation of L. monocytogenes organisms or heat-killed L. monocytogenes (HKL) with MΦ and NK cells results in ∼2-fold higher concentrations of IFN-γ compared with Listeria-stimulated MΦ and NK cells cultured in the same well but separated by a semipermeable membrane. The latter system allows free passage of cytokines but inhibits cell-to-cell contact between the two cell types. Moreover, in vitro experiments with Pneumocystis carinii show that IFN-γ is not produced by NK cells at all when these are separated by a semipermeable membrane from MΦ cultured with this extracellular fungal pathogen. However, when P. carinii organisms are given to nonseparated MΦ and NK cells, IFN-γ release can be observed at levels comparable with those produced in response to stimulation with L. monocytogenes (11). These results led to the hypothesis that cell contact-dependent costimulatory signals might also play a role in initiating cellular cooperation in natural immunity.

We approached this question in the in vitro listeriosis model and SCID WSC with functional inhibition studies using a panel of mAbs against different MΦ surface molecules. Incubation with an Ab directed against the murine MΦ surface glycoprotein F4/80 (12) resulted in markedly reduced levels of IFN-γ released into the supernatant (SN). Because of its highly restricted expression, the F4/80 Ag is well expressed by MΦ and NK cells, which lack functional T and B cells (3, 4). A number of MoAbs against different MΦ surface molecules in natural immune reactions to L. monocytogenes (HKL) with MΦ and NK cells

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2 Abbreviations used in this paper: WSC, whole spleen cells; MΦ, macrophages; HKL, heat-killed Listeria monocytogenes; iNO, inorganic NOs; iNOS, inducible NO synthase; SN, supernatant; HPRT, hypoxanthine phosphoribosyltransferase.

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Materials and Methods

Animals and microorganisms

Male and female C.B-17scid/scid (SCID) mice 6–8 wk old (21), provided by the Deutsches Krebsforschungszentrum (Heidelberg, Germany) and bred at the Zentrumeinheit, Zurich, and the Deutsches Krebsforschungszentrum (Heidelberg, Germany), were kept at the Robert Koch Institute in a separate animal room in filter-top cages within laminar air flow cabinets. All material, including water, bedding, and food was sterilized before use, and the filter tops were removed only within class II safety cabinets. The mice were negatively screened for serum Ab and were age and sex matched within individual experiments. The mouse-pathogenic L. monocytogenes strain EGD, serotype 1/2b (22), was raised in tryptone soya broth (36°C, 24 h; Oxoid, Basingstoke, U.K.), washed three times in HBSS (2000 × g, 6°C, 12 min), and counted as CFU on tryptone soya agar (Oxoid), and stored in aliquots at −70°C. For HKL, bacteria were exposed to 62°C in a water bath for 60 min. Before killing, viable bacteria were assessed as CFU and the concentration of HKL estimated accordingly. Aliquots of HKL were frozen in HBSS at −20°C until use.

Cells

For WSC preparations, spleens from SCID mice (six per experiment) were homogenized and red cells removed by hypotonic lysis. For purification of NK cells, adherent cells were removed by incubating WSC in nylon wool columns (VYGON, Lyon, France) as described (23). Purity of cells in the effluent was assessed according to morphologic, antigenic, and functional criteria. SCID mouse-derived, nonadherent cells of large granular lymphocyte morphology, negative for nonspecific esterase (Sigma, Deisenhofen, Germany) and F4/80, positive for asialo GM1 (Wako Chemicals, Neuss, Germany) (24, 25), and toxic for YAC-1 cells (26) were designated NK cells. Addition of low concentrations of IL-2 (10 U/ml; a concentration below activation threshold) was necessary to prevent cell death (apoptosis) of the NK cells. M5/114 (both hybridomas were kindly provided by the Deutsches Krebsforschungszentrum (Heidelberg, Germany) and bred at the Deutsches Krebsforschungszentrum (Heidelberg, Germany) and bred at the Deutsches Krebsforschungszentrum (Heidelberg, Germany).

RT-PCR

Total RNA was extracted from 5 × 10⁶ WSC with Trizol (Life Technologies, Karlsruhe, Germany) according to the manufacturer’s instructions. First-strand cDNA was synthesized from 1–5 μg total RNA by 200 U Superscript II reverse transcriptase (Life Technologies) with 100 ng random primers (Life Technologies). First-strand cDNA was amplified by PCR using 2 μM sense/antisense primers with 2.5 U Taq polymerase (Goldstar, Eurogentec, Seraing, Belgium) in a Genius Thermocycler (Gien, Thermo-Dux, Wertheim, Germany) in a total volume of 50 μl. The reaction buffer consisted of 75 mM Tris-HCL, 2.5 mM MgCl₂, 20 mM (NH₄)₂SO₄, 0.01% Tween 20, and 20 μM dNTP. Thirty-six PCR cycles were performed (1 min at 94°C, 1 min at 61°C, and 3 min at 72°C). First cycle denaturing was conducted for 2 min at 94°C, and a final extension was performed for 4 min at 72°C. The sense and antisense primers were as follows: IL-1β sense, 5′-GCAACTGTCTGTTAACTCA-3′; IL-1β antisense, 5′-CTGGACTCTGTATGAGC-3′; IL-10 sense, 5′-TACCTGTGAGAAGTGTAATCTG-3′; IL-10 antisense, 5′-CATGTGACCTTCAGCTG-3′; IFN-γ sense, 5′-CTTCACTGGAAGTGGTCCG-3′; IFN-γ antisense, 5′-TCAGGACGTCCTTCGTTGTCTGGC-3′; TNF-α sense, 5′-TACTGCTGGTGC-3′; TNF-α antisense, 5′-CTCTGGAAGATGTTGC-3′; IL-12 sense, 5′-CTTTACACTGGAAGTGGTCCG-3′; IL-12 antisense, 5′-CATGTGACCTTCAGCTG-3′; IL-6 sense, 5′-GATGCC-3′; IL-6 antisense, 5′-GATGCC-3′; iNOS sense, 5′-CAAGAGACTCAAAGCTGACG-3′; iNOS antisense, 5′-GAGGTAGCGAAGTGG-3′. RT-PCR products were electrophoresed through a 1.0% agarose gel containing 0.3 μl ethidium bromide with a 100 bp DNA marker (Life Technologies) run in parallel. The gel was read in a UV transilluminator and documented by photography (Biotex Göttingen, Germany). All samples of an experiment were first analyzed for HPRT mRNA only. The HPRT bands were compared densitometrically (WinCam Software, Cybertech, Berlin, Germany), and the samples were diluted accordingly to achieve a common initial cDNA concentration before being analyzed for cytokine message.

Detection of inorganic NOs (iNOs) and cytokines

iNOs were quantitated with Griess reagent according to Reference 28, as described elsewhere (29) (lower detection threshold, 5 μM). IL-1β was measured by ELISA using different polyclonal rabbit anti-mouse-IL-1β sera from different suppliers (Zwischenrein, Belgium) for coating and biotinylated rat anti-mouse IL-10 clone SXC-1 (IgM; Pharmingen) for detection (threshold, 40 pg/ml). Production of IL-10 was measured by ELISA using the rat anti-mouse-IL-10 clone JES.5-2A5.1.1 (IgG1; kindly provided by Greg Bancroft, London School of Hygiene and Tropical Medicine) for coating and biotinylated rat anti-mouse IL-10 clone SXC-1 (IgM; Pharmingen) for detection (threshold, 80 pg/ml). Production of IL-12 (p40/p70) was measured by ELISA using the rat anti-mouse clone C15.6 (IgG1) for coating and biotinylated C17.8 (IgG2a; both Pharmingen) for detection (threshold, 80 pg/ml).

Statistics

Significance of results was determined according to Student’s t test.

Results

Release of HKL-induced NK-derived IFN-γ is mediated by soluble Mφ- derived factors but is reduced when cell contact is inhibited

MΦ and NK cells are both necessary and sufficient for HKL particles to induce IFN-γ. As shown in Table I, the concentrations of IFN-γ released by equal numbers of these cell types differ according to the experimental design. The highest levels of IFN-γ are reached when HKL, MΦ, and NK cells are cocultivated in the
same well. When NK cells are cultivated in the SN of HKL-treated MΦ, IFN-γ release is appreciable, but reduced compared with the coculture system. To ensure that this reduced performance of NK cell activation via soluble cytokines, a costimulatory pathway might exist that requires close vicinity between MΦ and NK cells, possibly even direct cell-to-cell contact.

**Ab against the MΦ surface Ag F4/80 inhibits NK cell-derived IFN-γ release**

Assuming that cell contact promotes an important costimulatory mechanism in microbe-induced MΦ/NK cell interaction, HKL-stimulated SCID WSC were incubated with a panel of mAb directed against different MΦ surface molecules. As shown in Fig. 1, coincubation with mAb against the F4/80 molecule had a significant inhibitory effect on HKL-induced IFN-γ release, whereas mAb directed against other MΦ surface Ags or the IgG2b-isotype control had neither inhibitory nor enhancing effects.

**Table II. Effect of anti-F4/80 mAb on HKL-induced MΦ-mediated IFN-γ-production by NK cells**

<table>
<thead>
<tr>
<th>NK Cells Incubated with</th>
<th>HKL</th>
<th>MΦ, a not separated</th>
<th>MΦ, b separated</th>
<th>MΦ, c SN-transfer</th>
<th>α-F4/80 d (20 μg/ml)</th>
<th>IFN-γ e (ng/ml ± SD)</th>
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a Nylon wool-purified NK cells (5 × 10⁷/ml/well) were incubated as indicated for 48 h; one of four experiments with similar results is shown.

b MΦ and HKL were cultured together with NK cells.

c MΦ and HKL were cultured separated by a semipermeable membrane.

d NK cells cultured in SN from MΦ coincubated with HKL.

e As tested by ELISA; means of triplicates. * p < 0.0001 vs no anti-F4/80 mAb.
in a dose-dependent manner. Control isotype-matched mAb or anti-LFA mAb showed no significant effect. In contrast to its effect on IFN-γ, TNF-α, and IL-12, anti-F4/80 mAb enhanced release of IL-1β and IL-10. Synthesis of iNO, often used as an indicator of murine MΦ activation, was not modulated significantly. Interestingly, anti-F4/80-matched isotype control mAb R35–38 had similar, if lower, enhancing effects on the release of IL-1β and IL-10, whereas incubation with anti-LFA-1 mAb again had no effect. These data indicate that the modulatory effects of anti-F4/80 mAb on HKL-induced cytokine release by WSC differ according to the individual cytokine.

**Kinetics of cytokine release in HKL-stimulated SCID WSC and modulating effects of anti-F4/80**

For further elucidation of HKL-induced reaction of spleen cells in absence of functional T or B cells, the order of appearance of individual cytokines and the modulatory effects of anti-F4/80 were analyzed (Fig. 4). In the absence of anti-F4/80, IL-10, IL-12, and IFN-γ could be detected at 6 h, and iNO at 8 h, after addition of HKL. Concentrations of these three factors increased further or reached a plateau during the 72 h they were observed. TNF-α was detectable already after 2 h, peaked at 24 h, and then rapidly diminished. Constitutive levels of IL-1β were not modified by HKL; they remained low over the study period. For all factors, the effects of anti-F4/80 were detectable with their first appearance in the SN. The specific modulatory effects did not change in quality over time. Incubation with anti-F4/80 isotype control mAb had no significant effect on HKL-induced cytokine release, again with the exception of IL-10 and IL-1β, which were induced both by anti-F4/80 and by its isotype control.

Nonspecific Fc-receptor binding and signal transduction was deemed a likely explanation for the agonistic effects of anti-F4/80 mAb and its isotype control. Accordingly, non-Ag-specific binding of Ab was blocked by specific, inhibitory anti-FcγIII (CD16)/FcγII (CD32) mAb (Fc block) before adding test Ab and HKL to cell cultures. Whereas absence or presence of Fc block had no significant effect on inhibition of IFN-γ release by anti-F4/80 (2.12 ± 0.29 vs 2.09 ± 0.28 ng/ml; WSC + HKL alone, 5.03 ± 0.39), Fc block annulled both the agonistic effects of anti-F4/80 on IL-10 release (0.92 ± 0.18 vs 0.67 ± 0.18) and those of its isotype control (0.83 ± 0.21 vs 0.65 ± 0.19; WSC + HKL alone, 0.66 ± 0.15).

**Inhibitory effects of F4/80 mAb are specific and mediated by Fab fragment**

To further clarify the diverging effects of anti-F4/80 mAb on HKL-induced WSC cytokine release, Fab and Fc fragments of the Ab were tested individually. Fig. 5 illustrates that the inhibitory effect of F4/80 mAb was mediated by the Ag-specific Fab-fraction. Moreover, F4/80 Fab also inhibited induction of IL-10. On the other hand, the stimulatory effect of F4/80 mAb, specifically on IL-10 release, was mediated by its Fc fragment only. Release of IL-10 might be responsible for the marginal, nevertheless significant inhibition of cytokine release observed for Fc fragment alone.

**Inhibition of IFN-γ release in the presence of anti-F4/80 is only apparent when direct cell contact between MΦ and NK cells is possible**

As a first investigation into the mechanism by which anti-F4/80 modulates HKL-induced NK-derived IFN-γ release, different culture systems were employed, allowing or preventing direct contact...
between MΦ and NK cells. As shown in Table II, detectable amounts of IFN-γ were induced when HKL were given to cocultures of MΦ and NK cells, when HKL-stimulated MΦ and NK cells were separated by a semipermeable membrane, and when cell culture SN from HKL-stimulated MΦ was given to NK cells. HKL-stimulated cocultures revealed at least twice the amount of IFN-γ found in the other culture systems. Addition of anti-F4/80 mAb reduced IFN-γ release only in the coculture system. Anti-F4/80 mAb had no effect when direct cell contact between HKL-stimulated MΦ and NK cells was inhibited.

**FIGURE 3.** Anti-F4/80 mAb differentially modulates cytokine release in HKL-stimulated SCID WSC cultures. SCID WSC (1 × 10⁶/ml/well) were incubated with HKL (1 × 10⁷/well; dark gray) and anti-F4/80 mAb (filled), an IgG2b isotype control (light gray), or anti-LFA-1 mAb (open) at the concentrations indicated for 24 h (TNF) or 48 h (other factors), and the SNs were tested for cytokines by ELISA. Spontaneous release: IFN-γ, 0; IL-12, 0.12 ± 0.1; TNF-α, 0; iNO, 0; IL-1β, 0.24 ± 0.1; and IL-10, 0. A representative experiment of four is shown. Values in ng/ml (iNO in μM) express means of triplicates ± SD; *, p < 0.0001; **, p < 0.001 vs control.

**FIGURE 4.** Kinetics of cytokine release in HKL-stimulated SCID WSCs and modulating effects of anti-F4/80 mAb. SCID WSC (1 × 10⁶/ml/well) were incubated with HKL (1 × 10⁷/well) alone (open), with HKL plus anti-F4/80 mAb (12 μg/ml; filled), or HKL plus an IgG2b isotype control (12 μg/ml; gray) for the times indicated, and the SNs were tested for cytokines by ELISA. A representative experiment of four is shown. Values in ng/ml (iNO in μM) express means of triplicates ± SD; no asterisk, p > 0.0001; *, p < 0.05; **, p < 0.001; ***, not significant vs control.
Inhibitory effect of anti-F4/80 mAb is mediated by Fab fragment. SCID WSC (1 × 10^6/ml/well) were incubated with HKL (1 × 10^7/well) with either the Fab or the Fc fragment of anti-F4/80 mAb at the concentrations indicated for 24 h (TNF-α) or 48 h (other factors), and the SNs were tested for cytokines by ELISA. Spontaneous release: IFN-γ; 0; IL-12, 0.12 ± 0.1; TNF-α, 0; iNO, 0; IL-1β, 0.24 ± 0.1; IL-10, 0. A representative experiment of three is shown. Values express means of triplicates ± SD; *, p < 0.0001; **, p < 0.001 vs WSC + HKL alone.

**FIGURE 5.**

Discussion

In a previous publication we suggest that auxiliary, cell contact-dependent, pathways might be involved in microbe-induced, MΦ-mediated NK cell activation. For example, *P. carinii* organisms stimulate IFN-γ production in SCID WSC cultures only, when both MΦ and NK cells are present and cell contact is not inhibited (5). We now provide evidence that binding of the murine MΦ surface glycoprotein F4/80 with specific mAb modulates cytokine levels induced by HKL in SCID WSC or in cocultures of MΦ and NK cells. This report shows that in SCID WSC: 1) inhibition of HKL-induced IFN-γ, TNF-α, and IL-12 by anti-F4/80 mAb or Fab fragment is Ab specific and dose dependent; 2) this inhibition is apparent at mRNA and protein release levels; 3) modulation of cytokine release by anti-F4/80 is effective as soon as the cytokines are detectable in the SN; 4) anti-F4/80 only inhibits release of IFN-γ, TNF-α, and IL-12 when both MΦ and NK cells are present in the culture system; and 5) anti-F4/80 is only effective when cell contact between MΦ and NK cells is not inhibited. These data further indicate that an auxiliary microbe-induced signaling pathway between MΦ and NK cells may exist involving cell surface molecules.

The natural or innate immune system that includes potent effector cells such as MΦ, granulocytes, and NK cells is responsible for the early partial resistance to the facultative intracellular bacterial pathogen *L. monocytogenes* in SCID mice (30). Cytokine signaling plays a major role in activation of this T cell-independent immune response. MΦ respond directly to organisms of large taxonomic variety by releasing TNF-α and IL-12 (31–35). In combination, TNF-α and IL-12 activate NK cells for release of IFN-γ, which in turn augments MΦ microbicidal activity. This cytokine-mediated activation cycle is under negative control of IL-10, which inhibits IL-12 release by MΦ as well as IFN-γ production by NK cells in response to TNF-α plus IL-12 (5, 7, 36).

In a variety of experiments, however, we observed that soluble cytokine signaling might not cover all aspects of microbe-induced MΦ/NK cell interactions. First, stimulation of purified MΦ with *P. carinii* organisms, opportunistic fungal agents of pneumonia in severely immunocompromised individuals, does not lead to TNF-α or IL-12 release. Nevertheless, *P. carinii* induces maximum levels of IFN-γ in cocultures of MΦ and NK cells or in SCID WSC cultures similar to those of *L. monocytogenes* (5, 11). These data seem irreconcilable with the above-outlined model of NK cell activation by microbe-induced, MΦ-released cytokines. Second, the amount of IFN-γ induced by HKL depends on whether HKL-stimulated MΦ and NK cells are incubated together or are separated by a semipermeable membrane, with the former giving at least 2-fold higher concentrations of IFN-γ than the latter (Table 1). Initial experiments had shown that, with respect to the in vitro experiments presented here, viable *L. monocytogenes* organisms and HKL particles have a similar capacity for inducing cytokine release in SCID WSC or in purified MΦ and NK cell cocultures. Furthermore, anti-F4/80 mAb exerted the same inhibitory effects whether these cell cultures were stimulated with viable *L. monocytogenes* or with HKL.

These qualitative and quantitative deviations from the current model of a microbe-induced cytokine cascade led to the hypothesis that costimulatory signaling pathways may exist that require direct contact between MΦ and NK cells and act via cell surface molecules.

To examine this hypothesis, blocking experiments were performed with a panel of mAb for murine MΦ surface molecules added to SCID WSC in the presence of HKL (Fig. 1). Abs directed against the mouse MΦ surface glycoprotein F4/80 specifically inhibited HKL-induced, NK cell-derived IFN-γ. The remaining levels of IFN-γ were similar to those found when HKL-stimulated MΦ and NK cells were kept separated by a semipermeable membrane or when cell-free SNs from HKL-stimulated MΦ were given to purified NK cell populations (Table 1). Anti-F4/80 mAb is widely used for immunohistochemical or cytofluorometric detection of mouse MΦ. The mAb was tested for cytotoxic effects on MΦ or NK cells in MTT assays (27). No differences in cell viability in the absence or presence of anti-F4/80 mAb were observed for either cell population over 48 h (data not shown). As certain mAb have been reported to induce apoptosis (37), a cell death detection assay using the TUNEL method was employed to address this possibility. The results gave no indication for anti-F4/80-mediated apoptosis (data not shown).

Semiquantitative RT-PCR performed on anti-F4/80 mAb-treated and control cultures of HKL-stimulated SCID WSC revealed that inhibition of IFN-γ occurred already at pretranscriptional levels. Furthermore, mRNA expression of TNF-α and IL-12 was also inhibited, whereas IL-1β and IL-10 were enhanced, and iNOS mRNA was not obviously affected (Fig. 2). Cocultures in the presence of anti-F4/80 Fab showed similar inhibition of IFN-γ, TNF-α, and IL-12 mRNA expression, whereas IL-1β, IL-10, and iNOS were not biased. The inhibitory effects of anti-F4/80 were clearly concentration dependent (Fig. 3) and evident as soon as the respective cytokine was detectable in the SN (Fig. 4).
Release of certain cytokines that are not strongly induced by HKL under the given experimental conditions, such as IL-1 and IL-10, were enhanced when anti-F4/80 mAb was added (Fig. 2). This differential modulation of cytokine release by anti-F4/80 could be mediated by at least three different mechanisms. First, MΦ could be nonspecifically stimulated by anti-F4/80 mAb via their Fc receptors. Accordingly, nonAg-specific binding was blocked by preincubating cultures with specific, inhibitory anti-FcγII (CD16)/FcγII (CD32) mAb (Fc block). Whereas absence or presence of Fc block had no significant effect on inhibition of IFN-γ release by anti-F4/80, it annihilated the agonistic effects of anti-F4/80 on IL-10 release. We investigated the possibility of nonspecific effects of the anti-F4/80 mAb by separating its Fab from its Fc fragments. The Fab fragments inhibited TNF-α, IL-12, and IFN-γ production in HKL-stimulated SCID WSC cultures as described for the whole Ab (Fig. 5), indicating that these effects were Ag specific. On the other hand, only Fc fragment of anti-F4/80 enhanced release of IL-10 or IL-1β, as found for whole mAb, indicating stimulation of IL-10-, and IL-1 could indeed have been mediated nonspecifically by FcR binding. These data also show that inhibition of IFN-γ, TNF-α, and IL-12 by anti-F4/80 mAb did not result simply from enhanced IL-10 release. Direct effects of anti-F4/80 mAb on NK cells can probably be ruled out. When NK cells were stimulated with TNF-α plus IL-12, addition of anti-F4/80 mAb had no effect on the levels of IFN-γ produced (data not shown).

Second, specific binding of mAb to the F4/80 Ag might act indirectly by initiating signal transduction leading to down-regulation of as-yet-unknown costimulatory ligands or inducing production of molecules inhibitory for NK cell functions. Experiments performed to date give no indication for either pathway. For instance, release of TNF-α or IL-12 by HKL-stimulated MΦ alone was not altered in the presence of anti-F4/80 mAb nor was HKL-induced release of IL-10 or IL-1 affected by anti-F4/80 Fab. Analysis at the mRNA level gave the same results (data not shown). Apparently, the inhibitory activity of anti-F4/80 related only to MΦ/NK interaction and not to inducible functions of either cell population alone.

Third, specific binding of mAb to the MΦ surface Ag F4/80 might directly inhibit contact with an as-yet-unknown ligand on NK cells. With strong MΦ stimulants such as HKL that can directly induce TNF-α and IL-12 release, binding of F4/80 to this putative ligand may act as an additional stimulus, enhancing NK cell activation and IFN-γ release. With weak MΦ stimulants such as P. carinii organisms (5, 11), which do not directly induce TNF-α and IL-12 release, binding of F4/80 to its NK cell ligand is an essential step. Following a two-signal hypothesis for MΦ activation, the first signal (priming) would be delivered by adherence/phagocytosis of certain microorganisms. The second could either be delivered by other elements of the same microorganism (strong stimulants) or by accessory stimuli such as binding of F4/80 to its putative ligand. The subsequent activation cascade would then be controlled by mutual cytokine release as observed in both HKL or P. carinii-stimulated NK/MΦ cocultures. Of the three, the latter mechanism seems best supported by the available data.

References


