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Chloroquine inhibits T cell proliferation by interfering with IL-2 production and responsiveness

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SUMMARY

Chloroquine (Chl) is an anti-rheumatic drug that is widely used in the treatment of rheumatoid arthritis (RA). It seems that T cells are important in the pathogenesis of RA, but it is not known whether Chl acts via inhibition of T cell function. We here present evidence that Chl, just like cyclosporine A (CsA), inhibits T cell proliferation as induced with immobilized αCD3 MoAb in a concentration-dependent manner, at least partly through interfering with the production of IL-2 protein and the induction of IL-2 mRNA. Furthermore, Chl impedes the responsiveness of T cell clones to IL-2 since (1) the inhibition of αCD3 MoAb-induced proliferation by Chl could not be reversed by rIL-2 and (2) Chl directly blocks IL-2-driven proliferation of cloned T cells. Chl appeared to interfere with the internalization (50% inhibition) and degradation (total blockade) of rIL-2. Finally, the combination of Chl and CsA synergistically inhibited T cell proliferation. We conclude that Chl may inhibit functional properties of human T cells, although the drug is 100- to 1000-fold less potent than CsA in inhibiting T cell proliferation and IL-2 production, respectively. It is speculated that the *in vitro* effects of Chl might be relevant in explaining the anti-rheumatic effect of this drug in patients with RA.

Keywords rheumatoid arthritis anti-rheumatic drug(s) cytokines cyclosporine A

INTRODUCTION

Chloroquine (Chl) is a 4-aminoquinoline that is used as a disease modifying anti-rheumatic drug (DMARD) to suppress disease activity in rheumatoid arthritis (RA) and lupus erythematosus. Several controlled studies with Chl have shown that the drug is more effective than placebo in patients with RA [1–4]. We have demonstrated recently that Chl is as effective as the immunosuppressive drug cyclosporine A (CsA) in patients with RA of limited duration [5]. The mechanism of action of Chl in RA is poorly understood, but that of CsA is known to include inhibition of T cell activity (reviewed in [6-9]). T cells are thought to be crucially important in the pathogenesis of RA [10–12], and one might suggest that the efficacy of Chl in RA could be attributed to inhibition of T cell activity. Chl has been shown to inhibit the responsiveness of peripheral blood mononuclear cells (PBMC) to mitogens [13], the production of interleukin-1 (IL-1) by mononuclear cells [14], and the production of tumour necrosis factor by macrophages [15]. In a previous study, we showed that Chl inhibited the production of interferon-gamma (IFN-γ) by activated T cell clones, suggesting that Chl interferes with T cell activity in some way [16].

T cell activation is induced by triggering the T cell receptor. Activated T cells induce mRNA for IL-2 and produce IL-2 protein; secreted IL-2 successively induces T cell proliferation by binding to the IL-2 receptor (IL-2R) present on activated T cells [17,18]. The focus of the present study was to investigate the effects of Chl on T cell proliferation and on IL-2 production and responsiveness, being the most important determinants of T cell proliferation. In order to induce T cell proliferation, T cell clones were activated with immobilized αCD3 MoAb. It was questioned whether Chl might inhibit αCD3-induced T cell proliferation and whether Chl interferes with the production of IL-2 mRNA and IL-2 protein. Furthermore, the effects of Chl on the responsiveness of T cells to IL-2 were studied by measuring IL-2-induced proliferative responses. Finally, since IL-2-responsiveness of T cell clones is at least partly determined by internalization and intracellular degradation of IL-2, we investigated whether Chl affects these processes.

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MATERIALS AND METHODS

Cells

Synovial T cell clones (with $CD4^+/CD8^-$ phenotype) obtained

from synovial tissue of a RA patient were used. The cloning procedure has been described elsewhere in detail [19,20]. Briefly, fragments of synovial tissue, obtained during a synovectomy, were placed in 24-well tissue culture plates (Costar 3524, Cambridge, MA) in culture medium (Iscoves Modified Dulbecco's Medium (IMDM; GIBCO, Grand Island, NY)) containing 10% fetal calf serum (FCS; GIBCO), supplemented with antibiotics (penicillin 100 IU/ml, streptomycin 100 μg/ml; Boehringer Mannheim GmbH, Mannheim, FRG) and with 5% T cell growth factor (TCGF) [21]. Growing cells were separated from tissue fragments and cloned by limiting dilution using a feeder mixture with irradiated (3000 rad) peripheral blood mononuclear cells, OKT3-ascites (10^{-5} dilution) and TCGF (5% v/v). Cells were maintained in culture by restimulating them once in 10–14 days with a feeder cell mixture in the presence of TCGF (5% v/v). Jurkat leukaemic T cells were grown in RPMI-1640 (GIBCO) culture medium supplemented with 10% FCS. Peripheral blood mononuclear cells from healthy adult volunteers were obtained by centrifugation over Ficoll-Hypaque cushions.

Drugs

CsA (Sandoz Ltd, Basle, Switzerland) was dissolved in ethanol and was further diluted in culture medium containing 10% FCS. A stock solution of 40 μg/ml was stored at −20°C for 8 weeks at most. This stock solution was further diluted to the appropriate concentrations in culture medium. Chl (Rhône-Poulenc, Amsterdam, The Netherlands) was dissolved in culture medium. A stock solution of 4 mg/ml was stored at −20°C for 8 weeks at most and diluted to appropriate concentrations in culture medium.

Proliferation assays

Cloned T cells (2×10^5) were incubated in 96-well flat-bottomed microtitre plates (Greiner, Arnhem a/d Rijn, The Netherlands), coated with αCD3 MoAb (Cilag, Herenthals, Belgium) (0·1 μg/ml in phosphate-buffered saline (PBS)), in 200 μl culture medium, containing various concentrations of CsA and Chl, for 48 h at 37°C, 5% CO_2. In some experiments, rIL-2 (Cetus, Gouda, The Netherlands) was added to the culture medium in various concentrations (International Units (IU)) and cells were placed either on αCD3 MoAb-coated or on uncoated plates. Jurkat cells (2×10^5) were incubated in 96-well flat-bottomed microtitre plates without any stimulus in 200 μl culture medium in the presence or absence of various concentrations of Chl. Twenty-four hours later, tritiated thymidine was added. After 48 h the cells were harvested and thymidine incorporation was measured in a liquid scintillation counter.

Flow cytometry analysis

Flow cytometry was performed as described previously [19]. Cloned cells or PBMC were activated on αCD3 MoAb-coated (0·1 μg/ml) plates for 24 h in the absence or presence of Chl (12 μM). After washing twice with ice-cold PBS containing 1% (w/v) bovine serum albumin (BSA), 1×10^5 cells/sample were incubated with an excess of αCD3 MoAb or anti-IL-2R MoAb (TAC; p55-chain) or with subclass-matched irrelevant antibodies for at least 30 min on ice. After two washes with ice-cold PBS-1%BSA, cells were labelled with fluoresceïn isothiocyanate (FITC) conjugated goat anti-mouse IgG (1/40 dilution in PBS-1%BSA). Cells were washed twice with PBS-1%BSA, fixed with 1% paraformaldehyde and analysed using a FACStar (Becton Dickinson, Mountain View, CA).

IL-2 assay

IL-2 was measured using an inhibition radioimmunoassay, essentially as has been described for IL-1 [22]. Briefly, ^{125}I-labelled IL-2 was incubated with a non-saturating concentration of a rabbit αIL-2 monospecific polyclonal antibody and 100 μl test sample, to make IL-2–αIL-2 complexes competitively. The complexes were precipitated with goat–anti-rabbit antiserum in the presence of polyethylene glycol (BDH, Poole, UK) 5·6% and non-bound ^{125}I-IL-2 was removed. Radioactivity of the precipitate was determined in a gamma-counter. A standard curve was constructed by adding serial dilutions of cold IL-2. IL-2 concentrations in the samples were determined by interpolation from the standard curve. The sensitivity of the assay was 500 pg IL-2 per ml.

IL-2 mRNA analysis

Cloned T cells (3×10^6) were incubated with phorbol 12-myristate 13-acetate (PMA) (1·0 ng/ml) and ionomycin (Iono) (A23187) (100 ng/ml) for 5 or 9 h in the absence or presence of CsA (125 ng/ml) or Chl (6·25 μg/ml). PMA/Iono stimulation was chosen since this combination resulted in an optimal prodution of IL-2 mRNA after 5 and 9 h. The concentrations of CsA and Chl were chosen as the lowest concentrations that gave substantial inhibition of IL-2 production in the αCD3-stimulated T cell experiments.

Total cellular RNA was isolated using RNAzol (Cinna/Biotecx Labs, Houston, TX). First-strand cDNAs of the various samples were prepared using oligo- dT primers (GIBCO, BRL). PCR reactions (final volume: 0·04 ml) were run in PCR-mix containing 0·4 μl (25 mM) dNTPs (dCTP labelled with ^{32}P); 50 pmol (0·5 μl) of each primer (in the case of IL-2: 5′ GAA TGG AAT TAA TAA TTA CAA GAA TCC C 3′ for the 3′ region; and 5′ TGT TTC AGA TCC CTT TAG TTC CAG 3′ for the 5′ region; in the case of β-actin: 5′ GTG GGG CGC CCC AGG CAC CA 3′ for the 3′ region; and 5′ GTC CTT AAT GTC ACG CAC GAT TTC 3′ for the 5′ region); 1 μl of the synthesized cDNA; and 0·16 μl DNA Taq polymerase. Reactions were run on a programmable thermocycler (Pharmacia Biotechnology, Uppsala, Sweden) for 35 cycles. The PCR-products were fractionated by means of electrophoresis on a 1·5% agarose gel containing ethidium bromide. DNA contents were checked by visualizing the ethidium bromide staining under UV light. Further, the gel was air-dried and developed on an X-omat AR film (Kodak Company, Rochester, NY).

IL-2 internalization and degradation experiments

IL-2 internalization and degradation was analysed essentially as described by Fujii *et al.* [23]. Cells were harvested, washed twice and pelleted in glass tubes. Each tube contained $0{\cdot}5 \times 10^6$ cells. In order to achieve metabolic inactivity, the tubes were placed on ice for 60 min. Subsequently, 100 μl of PBS with bovine serum albumin (BSA) 1%, containing ^{125}I-IL-2 ($60\ (\pm 10) \times 10^3$ ct/min) was added to each tube. After 30 min incubation on ice, cells were washed twice with ice-cold PBS–BSA1%. After washing, $3{\cdot}2\ (\pm 1{\cdot}0) \times 10^3$ ct/min (5·4%) were still detectable. These counts were considered to represent

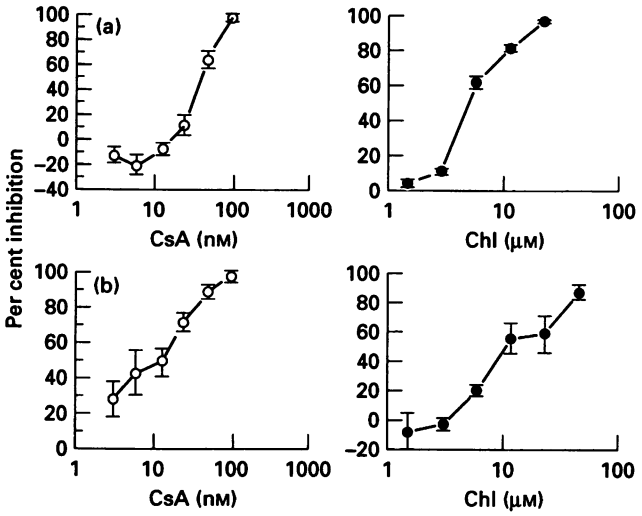

Fig. 1. Chloroquine inhibits αCD3 MoAb induced T cell proliferation and IL-2 production. Cloned T cells were stimulated with immobilized αCD3 MoAb (0·1 μg/ml) in the presence of CsA or Chl for 48 h, and proliferation was determined using thymidine incorporation (a). Cell-free supernatants were harvested 24 h after the initiation of the test and assayed for IL-2 (b). Results are expressed as mean (±s.e.m) percentage of inhibition relative to the drug-free situation.

membrane-bound IL-2 previous to internalization and degradation. Subsequently, cells were incubated at 37°C in 1 ml culture medium, containing 10^{-4} M Chl, for various time intervals in order to start internalization and degradation. Cells incubated in drug-free culture medium served as an internal control. The amount of non-specific IL-2 binding was determined by adding an excess of cold rIL-2. After the addition of an excess of cold rIL-2 (1000 IU/ml), 350 ct/min of non-specifically bound ^{125}I-IL-2 remained detectable. After incubation at 37°C the tubes were placed on ice instantaneously and the cells and their supernatants were separated by centrifugation. The supernatants were assayed for trichloric acid (TCA)-soluble radioactivity, that is a measure for degraded and exocytosed IL-2 [23].

The cell pellets were treated with 0·2 M glycine–HCl buffer (pH = 2.8) for 15 min (including centrifugation time) in order to strip membrane-bound non-internalized ^{125}I-IL-2. Cell free supernatants, containing previously membrane-bound ^{125}I-IL-2, were then separated from the cell pellets by centrifugation. Radioactivity was determined in the cell fractions (acid non-removable counts (ANRC)) and TCA-precipitable radioactivity was measured in the cell-free supernatants (acid removable counts (ARC)).

Calculations

In experiments designed to study the effects of drugs on T cell proliferation and IL-2 production, results are expressed as a percentage of inhibition relative to the drug-free situation:

$$\text{inhibition} = \left(1 - \frac{\text{proliferative activity or [IL-2] in the presence of the drug}}{\text{proliferative activity or [IL-2] in the absence of the drug}}\right) \times 100\%$$

Internalization of radiolabelled rIL-2 is expressed as a fraction of internalized radioactivity (ANRC), relative to the total radioactivity, bound before acid treatment (ANRC + ARC). IL-2 degradation was represented as the fraction of TCA-soluble counts in the supernatant relative to the total counts in the supernatant, collected directly after 37°C incubation. Synergy was determined as previously described in detail [24]. Briefly, isobolograms, with [CsA] on the horizontal axis and [Chl] on the vertical axis, were constructed by interpolating iso-effective concentrations from the concentration-effect curves. A straight line, representing the addition-isobole, was drawn connecting the concentrations of both drugs that represented the particular level of effect in the absence of the other drug. All iso-effective concentrations were then marked on the graph. When they all fall under the addition-isobole, the drug combination is considered to be synergistic [24].

RESULTS

Chloroquine inhibits T cell proliferation and IL-2 production by anti-CD3 MoAb-activated T cells

We first investigated whether Chl inhibits T cell proliferation, and which concentrations of Chl were required. Cloned T cells were activated with immobilized αCD3 MoAb in the presence of various concentrations of Chl or CsA in a 48 h proliferation assay. In these experiments maximum proliferative activity, measured without drugs, was approximately 35 000 ct/min. Background proliferative activity was 500 ct/min. Chl as well as CsA completely inhibited αCD3 MoAb-induced T cell proliferation in a concentration-dependent fashion as compared to the drug-free situation (Fig. 1a). The 50% inhibition concentration was approximately 7 μM for Chl and 70 nM for CsA, indicating that Chl is 100 times less potent than CsA in inhibiting T cell proliferation. The effects of both drugs were not due to cell death since fewer than 10% of the cells were trypan blue dye positive at the highest concentrations of both drugs.

We further studied the effects of Chl on the production of IL-2 by αCD3 MoAb-activated T cells. The maximum IL-2 production in these experiments was approximately 4·5 ng/ml/10^6 cells. Background IL-2 production was negligible. Chl and CsA both inhibited the production of IL-2 in a concentration-dependent fashion (Fig. 1b). The 50% inhibition concentration was approximately 11 μM for Chl and 11 nM for CsA, indicating that, based on equimolar concentrations, Chl is 1000 times less potent than CsA in inhibiting IL-2 production.

Chloroquine inhibits IL-2 production by interfering with the production of IL-2 mRNA

Since Chl was shown to inhibit the production of IL-2 by αCD3 MoAb-stimulated T cells, we compared the effects of Chl with those of CsA on the induction of IL-2 mRNA. Chl, like CsA, was able to inhibit the induction of IL-2 mRNA in stimulated T cells (Fig. 2). The induction of β-actin, which served as a control, was not influenced by CsA nor by Chl. These results indicate that Chl inhibits T cell proliferation by interfering with an early step in T cell activation.

Chloroquine inhibits the responsiveness of cloned T cells to IL-2, but does not affect IL-2-receptor expression

To investigate the precise site of action of Chl in T cell

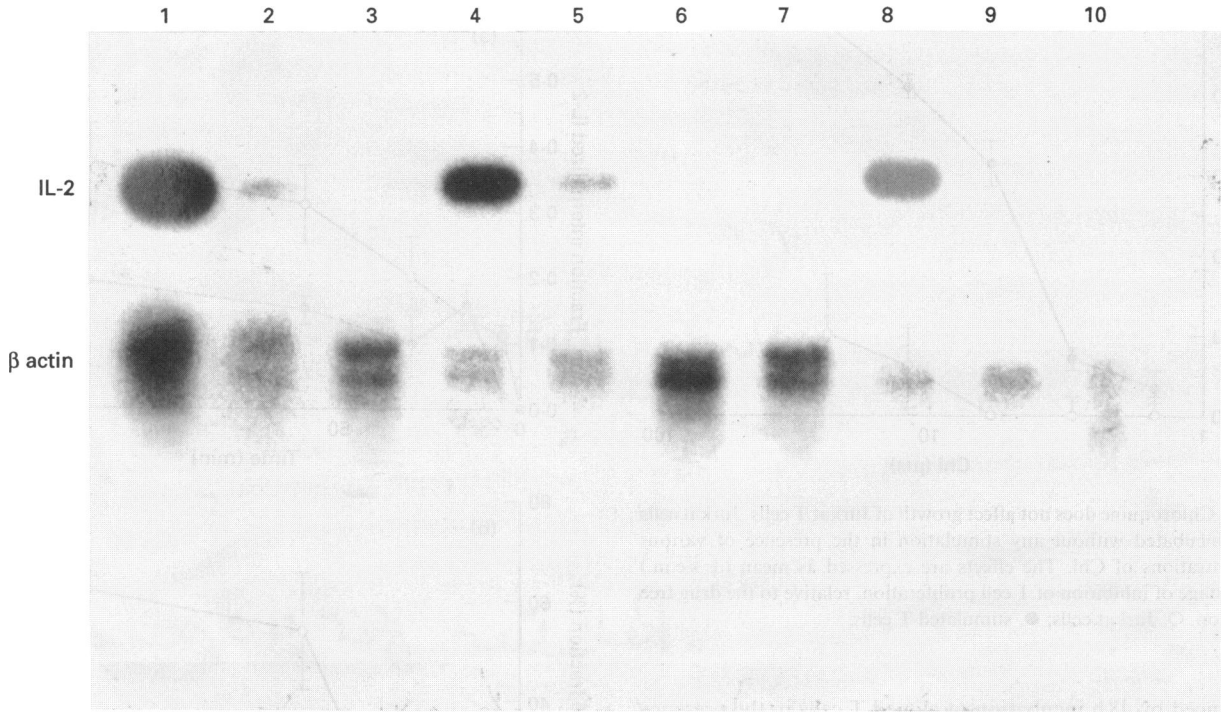

Fig. 2. Chloroquine inhibits the induction of IL-2 mRNA in cloned T cells. Cloned T cells were stimulated with PMA (1·0 ng/ml) and Iono (100 ng/ml) for 5 and 9 h in the presence or absence of CsA or Chl. IL-2 cDNA was specifically amplified using PCR with ^{32}P-labelled dCTP. One of three identical experiments is shown. Lanes 1 and 2: positive and negative control. Lanes 3 to 6: $t = 5$ h (lane 3, unstimulated; lane 4, stimulated; lane 5, stimulated with Chl; and lane 6, stimulated with CsA). Lanes 7 to 10: $t = 9$ h (lane 7, unstimulated; lane 8, stimulated; lane 9, stimulated with Chl; and lane 10, stimulated with CsA).

proliferation, cloned T cells were activated with αCD3 MoAb in the presence of various concentrations of rIL-2 (0–100 IU/ml) and in the presence of Chl or CsA. The addition of various concentrations of rIL-2 to the cells, stimulated with αCD3 MoAb in the presence of CsA, resulted in a concentration-dependent restoration of proliferative activity to the level of drug-free αCD3 MoAb induced proliferative activity (Fig. 3a). The effect of Chl, on the other hand, could not be overcome by the addition of an excess of 100 IU/ml rIL-2 (Fig. 3a).

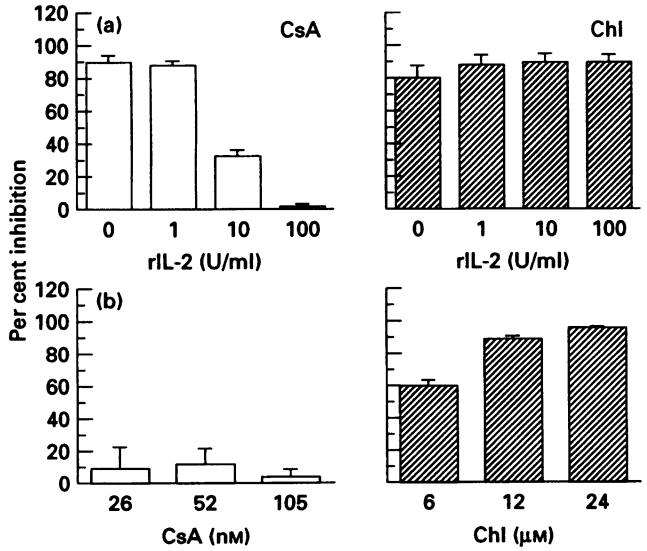

Fig. 3. Chloroquine inhibits the responsiveness of T cell clones to recombinant IL-2. Cloned T cells were stimulated either with immobilized αCD3 MoAb in the presence of CsA (53 nM) or Chl (12 μM) and in the presence of various concentrations of rIL-2 (a) or with rIL-2 (20 IU/ml) alone in the presence of various concentrations of CsA or Chl (b).

To investigate further whether Chl inhibits αCD3 MoAb induced T cell proliferation by interfering with IL-2 responsiveness, cloned T cells were stimulated with rIL-2 (20 IU/ml) alone on uncoated plates in the presence or absence of Chl or CsA. Stimulation with rIL-2 induced a high level of proliferative activity ($50 \pm 4{\cdot}5 \times 10^3$ ct/min (mean ± s.e.m.)). Chl completely prevented rIL-2 induced T cell proliferation in a concentration-dependent fashion (Fig. 3b). The 50% inhibition concentration was approximately 4 μM. CsA did not inhibit rIL-2 induced T cell proliferation using concentrations that were able to completely suppress αCD3 MoAb-induced proliferation (Fig. 3b).

The reversibility of the CsA-induced inhibition of T cell proliferation by rIL-2 proves that CsA is not toxic to the cells and that inhibition of IL-2 production by CsA is responsible for the effect of CsA on T cell proliferation. The toxicity of Chl was tested by studying the IL-2-independent growth of the Jurkat leukaemic T cell line in the presence of Chl. The growth of Jurkat cells was not significantly inhibited by concentrations of Chl that partially or completely blocked the αCD3 MoAb induced proliferation of clonal T cells (Fig. 4).

To investigate whether inhibition of IL-2 responsiveness by Chl is caused by inhibition of the expression of the IL-2R, the expression of the IL-2R p55 (Tac) chain was determined on cells incubated in the presence of Chl. Tac-expression was not

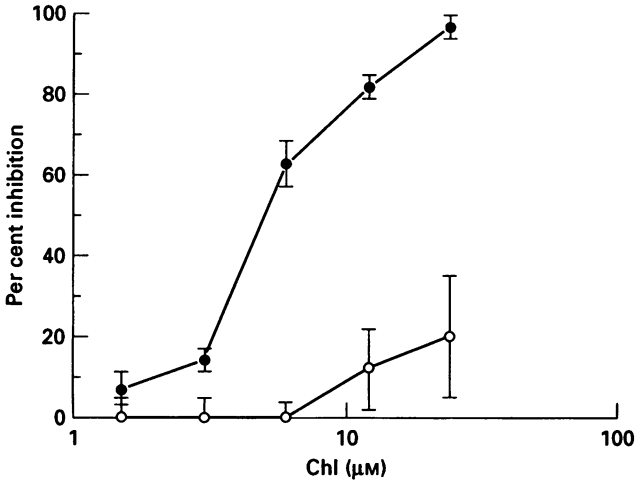

Fig. 4. Chloroquine does not affect growth of Jurkat T cells. Jurkat cells were incubated without any stimulation in the presence of various concentrations of Chl. The effects are expressed as mean (± s.e.m.) percentage of inhibition of T cell proliferation, relative to the drug free situation. O, Jurkat cells; ●, stimulated T cells.

influenced by 48 h incubation of cloned T cells in the presence of Chl (12 μM) (results not shown). To investigate whether Chl influences the *de novo* expression of the IL-2R on resting cells, PBMC were incubated in the presence of Chl during stimulation. Chl did not inhibit or enhance the induction of the expression of IL-2R on stimulated PBMC (results not shown).

Chloroquine interferes with IL-2 internalization and degradation

IL-2 when bound to its receptor is subsequently internalized and intracellularly degraded [23, 25]. Internalization and degradation of IL-2 are of pivotal importance in the induction of T cell proliferation [26]. Since it was found that Chl interferes with IL-2 responsiveness, we analysed the effect of Chl treatment on the process of IL-2 internalization and degradation. Cloned T cells were able to internalize ^{125}I-IL-2, as demonstrated by an increase of the fraction of internalized counts, reaching a plateau phase after 120 min incubation at 37°C (Fig. 5a). However, Chl-treated cells were not able to internalize as efficiently as non-treated cells, as demonstrated by a lower fraction of internalized counts after 60 min and 120 min. In order to investigate the influence of Chl on IL-2 degradation, the cell free supernatants collected after incubation were assayed for TCA-soluble radioactivity. Non-treated cells did not show TCA-soluble radioactivity (< 5%) in their supernatants up to 30 min incubation. After 60 min and 120 min incubation, TCA-soluble radioactivity rapidly increased (> 50% at 120 min), implying that the degradation of ^{125}I-IL-2 had started (Fig. 5b). Chl-treated cells did not show any increase in TCA-soluble radioactivity over the total time period analysed, suggesting that Chl-treated cells do not degrade ^{125}I-IL-2 (Figure 5b).

Combinations of chloroquine and cyclosporine synergistically inhibit αCD3 MoAb-induced T cell proliferation

Since CsA primarily inhibits IL-2 production, and Chl interferes with IL-2 responsiveness, both drugs might theoretically supplement each other's effects on T cell proliferation. To

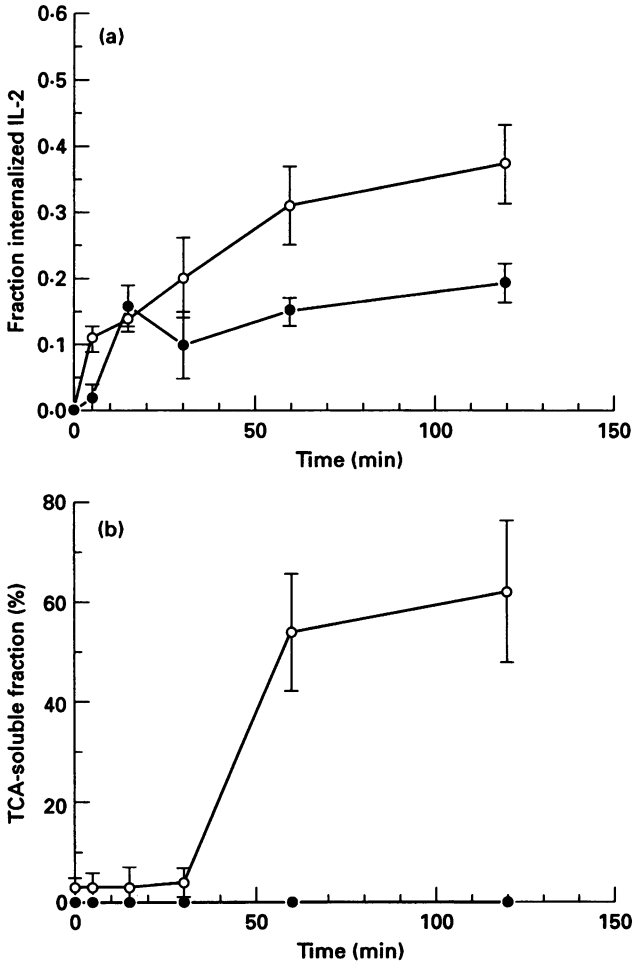

Fig. 5. Chloroquine inhibits internalization and intracellular degradation of ^{125}I-IL-2 by cloned T cells. Cloned T cells were saturated with ^{125}I-IL-2 and incubated at 37°C for various time intervals in order to start internalization and degradation of IL-2. Results are expressed as the mean (± s.e.m) internalized fraction of ^{125}I-IL-2 relative to the total amount of ^{125}I-IL-2, present before acid treatment (a), and as TCA-soluble fraction of radioactivity (as measure for degradation of ^{125}I-IL-2) in the supernatants (b). O, Non-treated cells; ●, Chl-treated cells.

test whether such a supplementary effect could be demonstrated, cloned T cells were activated with immobilized αCD3 MoAb in the presence or absence of various concentrations of Chl and/or CsA. The effects of drug combinations were studied in experiments set up in checkerboard fashion [24]. Drug concentrations that when tested alone demonstrated minor inhibitory effects on T cell proliferation, when used in combinations surpassed the sum of effects of both drugs separately (Fig. 6a), especially if CsA concentrations were used that exhibited a minor effect on T cell proliferation by themselves. In this situation, the addition of low concentrations of Chl provided significant potentiation of the inhibitory effect by CsA on αCD3 MoAb induced T cell proliferation. In the same fashion, addition of low concentrations of CsA to a low concentration of Chl did potentiate the inhibitory effect of Chl alone (Fig. 6a).

Because simple summation of separate effects is not sufficient to determine the character of this drug interaction [24],

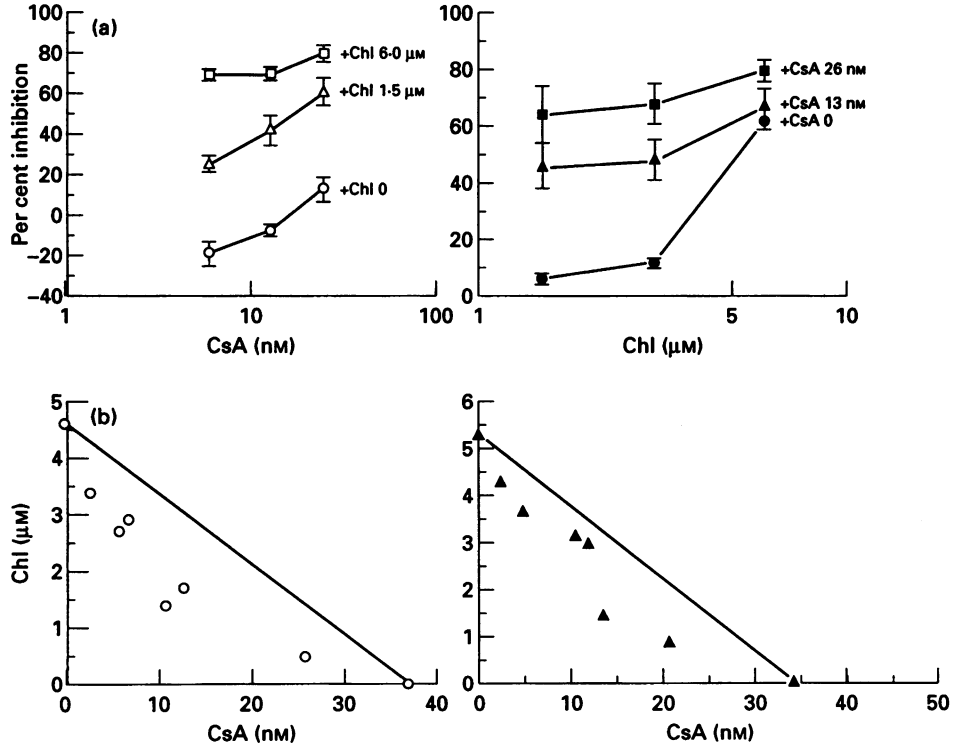

Fig. 6. Cyclosporine and chloroquine synergistically inhibit αCD3 MoAb induced T cell proliferation. Cloned T cells were stimulated with immobilized αCD3 MoAb in the presence or absence of CsA and Chl alone or in the presence or absence of combinations of CsA and Chl (a). Results are expressed as the mean (± s.e.m.) percentage of inhibition, relative to the drug-free situation. Synergy is determined by the construction of isoboles. The 40% effect isobologram (open symbols) and the 50% effect isobologram (closed symbols) are shown (b).

equipotent drug concentrations were calculated and isobolograms were constructed (Fig. 6b) [24]. The equipotent concentrations of CsA and Chl for 40% inhibition and for 50% inhibition, calculated from our experiments, are depicted under the straight-line addition isobole, demonstrating that 40% or 50% inhibition of αCD3 MoAb-induced T cell proliferation can be achieved by synergistically interacting concentrations of CsA and Chl.

DISCUSSION

The main conclusion of this study is that Chl inhibits IL-2 production as well as IL-2 responsiveness in T cell clones. The question whether the effects of Chl that have been demonstrated *in vitro* are relevant for the clinical situation is difficult to answer. Though the results demonstrate that Chl can inhibit T cell proliferation and IL-2 production, Chl is, based on equimolar concentrations, 100 to 1000 times less potent than CsA. However, it has been reported that Chl seriously accumulates in leucocytes, reaching intracellular concentrations that are 100 times higher than corresponding plasma concentrations [27]. Concentrations of Chl used in this study might therefore resemble intracellular leucocyte concentrations that are potentially achievable under therapeutic conditions.

Apart from the inhibition of IL-2 production and IL-2 mRNA induction, Chl was shown to modulate the IL-2 responsiveness of T cell clones. IL-2 production by αCD3 MoAb activated T cells is thought to be regulated in an autocrine fashion [17, 28]. αCD3 MoAb activate the production of IL-2, and IL-2 induces T cell proliferation via the interaction of IL-2 with the (high affinity) IL-2R. Although Chl did not influence expression levels of the IL-2R p55 chain, it cannot be excluded that the drug might affect some other component of the IL-2R complex [29]. The interaction between IL-2 and its receptor subsequently leads to enhancement of IL-2 production [28]. Therefore, inhibition of IL-2 production and inhibition of T cell proliferation can be the result of either abrogation of the activating signal or inhibition of IL-2 responsiveness.

The present study demonstrated that Chl partially inhibits the internalization, and totally prevents the intracellular degradation of IL-2. The difference in sensitivity of both processes to Chl indicates that Chl acts on both mechanisms, and that abrogation of degradation is not only caused by an impairment of IL-2 internalization. It has been demonstrated recently that IL-2 is internalized into the cell by receptor-mediated endocytosis, as has been described for other receptor systems [23, 25]. Prevention of endocytosis results in abrogation of the functional activity [26]. It has been suggested that degradation of IL-2 occurs in lysosomes and could be influenced by lysosomotropic agents such as Chl [23].

Chl is known to influence the behaviour of lysosomes by interfering with the vesicle fusion process in the cell [30], probably because of inhibition of lysosomal phospholipases [31]. This results in morphological changes essential for cell metabolism [32]. Cells treated with Chl are not able to proceed with endocytosis, exoplasmosis and phagolysosomal fusion

in an ordered manner [33]. This leads to sequestration of cellular membranes within the cell as the result of an arrest in normal recycling of vesicle membranes to the cell membrane; many cell receptors are thought to be involved in this sequestration process [34]. The effects of Chl on internalization and degradation of IL-2 are compatible with these suggestions. Similar effects of Chl on mouse T cells and PBL that confirm the results presented in this paper have been published recently [35]. It may be that part of the effect of Chl on cloned T cells has to be ascribed to functional abrogation of the activating αCD3 MoAb signal [36]. Abrogation of the activation signal might explain the inhibition of IL-2 mRNA production by Chl.

The demonstration of synergism in the inhibition of T cell proliferation between Chl and CsA further strengthens the observation of Chl-induced modulation of receptor-mediated endocytosis. CsA specifically inhibits the production of IL-2 by preventing the induction of mRNA for IL-2. Chl may prevent the induction of T cell proliferation by small amounts of IL-2, being produced by stimulated T cells in the presence of low concentrations of CsA. Such a mechanism resembles the synergy that has been demonstrated between CsA and monoclonal antibodies directed against the IL-2R [37, 38].

In conclusion, the present study has supplied evidence that the anti-rheumatic drug Chl influences crucial properties of activated T cells such as IL-2 production and IL-2 responsiveness. The mechanism of action of Chl structurally differs from that of CsA, although both drugs affect T cell function. The differences in mechanism of action might be important for the application of both drugs in the clinical situation, because of their potential to exert synergistical interactions.

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