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Identification of human IgG autoantibodies specific for IL-10

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SUMMARY

Since autoantibodies to IL-1α, interferon-alpha (IFN-α) and IL-6 have been described, this study concentrated on the search for autoantibodies to hIL-10 using an assay based on the precipitation of ^{125}I-hIL-10 anti-IL-10 autoantibody complexes using Protein G-Sepharose. Among 1860 tested sera, only seven were found to specifically precipitate IL-10, thus indicating the rare occurrence of such autoantibodies. Four of those seven anti-IL-10 autoantibody sera were specific for hIL-10, two recognized both human and viral IL-10, while the last one recognized human, viral and murine IL-10, thus suggesting the existence of at least three different epitopic specificities. The purification of anti-IL-10 autoantibody from one serum demonstrated the existence of a single (IgG1, λ) autoantibody that neutralized IL-10 biological activity. Thus, autoantibodies to IL-10 may represent natural antagonists to IL-10.

Keywords autoantibodies IL-10 human

INTRODUCTION

IL-10 is a cytokine produced by activated T and B lymphocytes, monocytes/macrophages and keratinocytes [1,2]. *In vitro*, human IL-10 (hIL-10) enhances humoral type responses by enhancing B cell viability [3] as well as stimulating the proliferation and differentiation of activated B cells [4]. However, IL-10 alters cellular immune responses by inhibiting the presentation of antigen to T cells by monocytes [5] and dendritic cells [6]. Interestingly, an open reading frame (BCRF-1) in the genome of the Epstein–Barr virus displays remarkable homology to hIL-10 [7,8]. The protein encoded by the BCRF-1 gene (vIL-10) shares a number of biological activities of hIL-10 [7].

The potent immunomodulatory effects of hIL-10 suggest that its actions must be tightly regulated *in vivo*. In this context, interferon-gamma (IFN-γ) has been demonstrated to efficiently inhibit IL-10 secretion by monocytes [9]. Other possible mechanisms of regulation may include the synthesis of specific inhibitors that could inhibit the binding of IL-10 to its cellular targets. Such an antagonist may be (i) a soluble form of the receptor (such as tumour necrosis factor (TNF) receptors for TNF-α and TNF-β); (ii) a specific antagonist of hIL-10 (such as IL-1RA for IL-1α); or (iii) a neutralizing antibody specific for hIL-10. This latter possibility should be considered, inasmuch as naturally occurring high-affinity and highly specific antibodies to cytokines such as IL-1α, IL-6, and IFN-α have been described in sera of healthy donors as well as sick patients [10–13]. In contrast, autoantibodies against denaturated cytokines have also been described (for IL-2, IL-8 and TNF-α) [14,15]. More recently, we have been able to isolate a human B cell line that secretes a high affinity (Kd = 10^{-10} M) neutralizing antibody specific to IL-1α [16]. Isolation and sequencing of the heavy and light chain mRNA demonstrated the presence of somatic mutations indicating antigen-driven affinity maturation [16].

In this study, we analysed the presence of anti-IL-10 autoantibodies in human serum and found that rare individuals display specific anti-IL-10 autoantibodies of IgG class. At least three different epitopic specificities could be identified, as demonstrated by their eventual reactivity with viral and murine IL-10.

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MATERIALS AND METHODS

Serum samples

Sera or plasma from healthy individuals and patients suffering from different pathologies such as autoimmune diseases (dermatitis, various chronic arthritis including rheumatoid arthritis (RA), systemic lupus erythematosus (SLE), myasthenia gravis), lymphoma, malaria, IgA deficiencies and enterocolitis, were tested for their ability to recognize iodinated hIL-10. In order to avoid degradation of cytokines and antibodies, all plasma and sera

were collected after venous blood punctions and immediately stored at –20°C.

Cytokines, cell lines and antibodies

Purified hIL-10 ($2{\cdot}1 \times 10^7$ U/mg) and hIL-4 (10^7 U/mg) (batch 8ILE1002) and IL-1α from chinese hamster ovary (CHO) supernatant were obtained from Schering Plough Research Institute (Kenilworth, NJ) (purity > 99%). Concentrated Cos7 transfection supernatants served as a source of viral IL-10 (vIL-10; 5 μg/ml) and murine IL-10 (mIL-10; 8·5 μg/ml) (provided by K. Moore, DNAX, Palo Alto, CA). The 19B9 cell line supernatant, used as source of mIL-3, was kindly provided by J. S. Abrams (DNAX).

Ba/F3 cells transfected with the human IL-10 receptor were provided by K. Moore and maintained in RPMI 1640 supplemented with 10% fetal calf serum (FCS) + 50 μM β_2-mercaptoethanol (β_2-ME) + G418 (GIBCO Labs, Grand Island, NY) and a mIL-3-containing supernatant of 19B9 cell line. The rat anti-hIL-10 MoAb (19B1) was a gift of J. S. Abrams (DNAX).

Immunoprecipitation of iodinated human IL-10

Purified hIL-10 was iodinated according to the Iodo-Beads iodination technique proposed by the manufacturer. Briefly, 10 μg of hIL-10 were added to Iodo-Beads (Pierce, Rockford, IL) previously incubated for 10 min with 500 μCi of $Na^{125}I$. After 15 min incubation, iodinated hIL-10 was separated from free ^{125}I on a Sephadex G25 PD10. Fractions with the highest quantity of radioactivity were pooled and tested. The specific activity of the labelled hIL-10 was $5–6 \times 10^4$ ct/min per ng. Under non-reducting conditions, the iodinated hIL-10 migrated at the same position as the bioactive non-iodinated cytokine (data not shown). ^{125}I-hIL-10 was tested for its capacity to bind to its receptor. Ba/F3 cells transfected with the hIL-10 receptor (hIL-$10R^+$ Ba/F3 cells) or only with the neo-R gene were used as target. So, ^{125}I-hIL-10 was able to bind to the hIL-10R + Ba/F3 cell line, whereas no binding was observed on neo-R Ba/F3 cells. So the iodination did not modify the capacity of the protein to recognize its receptor.

The presence of human autoantibodies (IgG) against hIL-10 in biological samples (sera or plasma) was determined using an immunoprecipitation assay with iodinated hIL-10 and Protein G-Sepharose which recognized all human IgG subclasses. Sera (50 μl) or plasma from patients (diluted in PBS–bovine serum albumin (BSA) 1%) were incubated for 45 min with 50 μl ^{125}I-hIL-10 (20 000 ct/min per condition) diluted in PBS–BSA 1% in a well of a flat-bottomed 96-well filtration microplate Multiscreen HA (Millipore Co., Bedford, MA). Then 50 μl of Protein G-Sepharose were added in each well and incubated for 45 min. Each sample was tested in duplicate. The wells were washed three times with PBS using a vacuum manifold (Millipore) and the dried membranes were collected. The radioactivity corresponding to the complexes ^{125}I-hIL-10–anti-hIL-10 was measured using a γ counter (Wizard; Wallac Co., Turku, Finland). Positive and negative controls were performed in each plate using, respectively, rat anti-hIL-10 antibody (19B1) and an unrelated antiserum. Specificity was further determined by preincubation of samples with a 100-fold molar excess of either unlabelled CHO cell-derived purified hIL-10, vIL-10 or mIL-10 produced by COS-7 cells.

In order to identify the isotype of anti-IL-10 autoantibodies contained in semi-purified immunoglobulin from patients' sera, the following precipitating agents were used: Affigel 10 (BioRad Labs, Richmond, CA) coupled with mouse MoAbs to human IgG1, IgG2, IgG3, IgG4 heavy chains (Calbiochem Co., La Jolla, CA) or with specific goat antibodies to human λ light chain or κ light chain (Sigma Chemical Co., St Louis, MO) according to the manufacturer's instructions.

To determine the sensitivity of this immunoprecipitation technique, a rat anti-hIL-10 MoAb (19B1) was diluted and the immunoprecipitation assay was performed as described above. The specificity was determined by preincubation of 19B1 with a 100-fold molar excess of cold hIL-10, vIL-10 and mIL-10.

Ammonium sulphate precipitation of positive plasma

Immunoglobulins from positive plasma were precipitated with 2·4 M ammonium sulphate for 1 h. After centrifugation, the precipitate was dissolved in PBS and dialysed at 4°C against large amounts of PBS to remove ammonium sulphate.

Preparation of immobilized hIL-10 and hIL-4

Recombinant hIL-4 and purified hIL-10 were coupled to Affigel 10 according to the manufacturer's instructions. Briefly, 2 mg purified hIL-10 (1 mg/ml) mixed with ^{125}I-hIL-10 (10^6 ct/min) were added to 2 ml Affigel 10. After 12 h of rotation at 4°C, unreacted binding sites were blocked with 200 μl Glycine Ethyl Ester pH 8. After 1 h incubation at room temperature, the gel was washed twice with deionized water and equilibrated in PBS–0·2% sodium azide. The coupling efficiency calculated from radioactivity incorporated into the gel was 92%.

Purification of human anti-hIL-10 autoantibodies

hIL-10-Affigel 10 (2 ml) was packed into a 10 ml Econocolumn (BioRad). Ammonium sulphate-precipitated and dissolved immunoglobulins from positive plasma were added and after 12 h rotation at 4°C, flow-through was collected and the gel was washed five times with 2 ml PBS. Then, the anti-hIL-10 autoantibodies were eluted with 0·1 M glycine buffer pH = 2·7 (2 ml/fraction) and immediatly neutralized using Trizma Base (1 M, pH 12, 100 μl/fraction).

Biological activity: hIL-10 assay

The Ba/F3 cell line transfected with the hIL-10 receptor (hIL-$10R^+$ Ba/F3 cells) proliferates in the presence of hIL-10 [17]. Their responsiveness to hIL-10 was tested as follows [18]. Briefly, cells were washed three times in medium to remove mIL-3 (used to expand the cells). Then, 10^4 cells were distributed in 96-well round-bottomed tissue culture plates (Nunc, Roskilde, Denmark) in 100 μl complete medium. They were incubated for 48 h with increasing concentrations of purified hIL-10 (up to 40 ng/ml). Cells were pulsed with ^3H-TdR for the last 18 h, harvested and counted. Tests were performed in triplicate and results were expressed as mean ct/min ± s.d.

The ammonium sulphate-precipitated and dissolved immunoglobulins of positive plasma, hIL-10-Affigel 10 flow-through or eluate were tested in this assay to determine the ability of anti-hIL-10 autoantibodies to inhibit hIL-10-induced cell proliferation.

RESULTS

Detection of anti-IL-10 autoantibodies by immunoprecipitation assay

Special care was taken to design an assay for anti-IL-10 IgG autoantibodies that would preferentially detect high-affinity

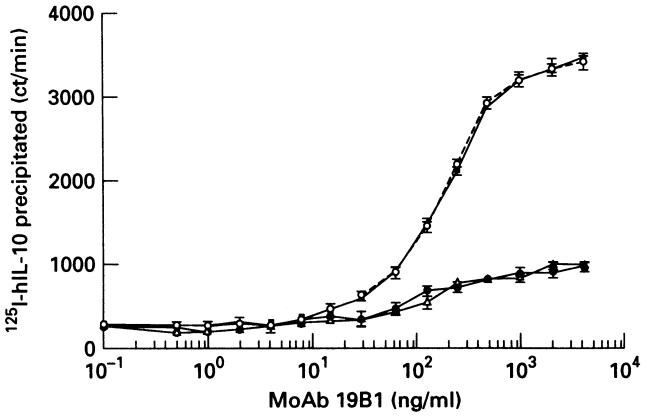

Fig. 1. Specific precipitation of ^{125}I-hIL-10 with the 19B1 rat anti-IL-10 antibody. Increasing concentrations of the rat MoAb (19B1) were incubated for 45 min with ^{125}I-hIL-10 (20 000 ct/min) and the complexes were immunoprecipitated with Protein G-Sepharose 4B (■). The specificity of the assay is demonstrated by preincubation of 19B1 with a 100-fold molar excess of hIL-10 (△), viral IL-10 (●) or murine IL-10 (○).

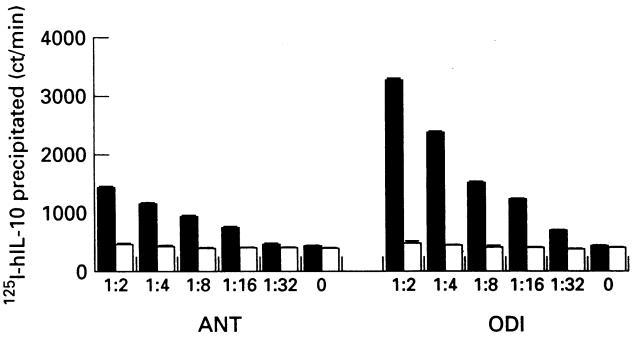

Fig. 2. Titres of anti-IL-10 autoantibodies in two positive sera. Serial dilutions (1:2–1:32) of positive anti-IL-10 sera (ANT, ODI), tested in duplicate were preincubated with medium (■) or a 100-fold molar excess of cold hIL-10 (□) for 20 min. Then, 50 μl of ^{125}I-hIL-10 (20 000 ct/min) were added and incubated for 45 min. Complexes were immunoprecipitated with 50 μl of Protein G-Sepharose 4B for 45 min. Free ^{125}I-hIL-10 was eliminated by washing and duplicates were counted using a γ counter.

antibodies and not antibodies of low affinity because of their memory B cell origin. This resulting screening is based on the immunoprecipitation of IgG bound to ^{125}I-IL-10 by Protein G-Sepharose.

To calibrate this technique and to determine the activity of the ^{125}I-hIL-10, a rat MoAb of IgG class, 19B1, was used, as it recognizes both hIL-10 and vIL-10 [19], but not mIL-10. Dilutions of this antibody were incubated with ^{125}I-hIL-10 (20 000 ct/min) and the complexes were precipitated with Protein G-Sepharose. As shown in Fig. 1, the anti-IL-10 MoAb (19B1) precipitates ^{125}I-hIL-10 in a dose-dependent manner with a plateau observed at $\approx$1 μg/ml and a limit of detection of 30 ng/ml. The specificity of this assay was demonstrated by the inhibition observed in the presence of a 100-fold molar excess of cold hIL-10 as well as vIL-10, but not with a 100-fold molar excess of mIL-10.

Table 1. Occurence of anti-IL-10 autoantibodies in human sera

Donors	Incidence	Seric hIL-10
Healthy donors	0/133	–
Chronic inflammatory arthritis	3/400	+
Pemphigus/pemphigoid	4/300	+
Myasthenia gravis	0/100	ND
Lupus erythematosus	0/45	ND
Lymphoma	0/58	+ + +
Malaria	0/130	+ + +
Crohn's disease/enterocolitis	0/42	ND
IgA deficiencies	0/16	–

The presence of anti-IL-10 autoantibodies was determined in sera from healthy donors as well as patients suffering from various pathologies. Sera (1 : 10 diluted) were incubated with ^{125}I-IL-10 (20 000 ct/min) and complexes were immunoprecipitated with Protein G-Sepharose. Seric hIL-10 levels were determined (when possible) using a double sandwich ELISA assay. Positivity was classified as negative sera (–), rare positive sera (+) and frequent positive sera (+ + +).

Rare human sera contain autoantibodies specific for IL-10

Detection of naturally occurring autoantibodies to hIL-10 in sera or plasma was performed using the radioimmunoprecipitation assay. Blood samples ($n = 1860$) from healthy donors or from patients suffering from various pathologies were screened in this assay at a 1:10 dilution. None of the sera from healthy donors ($n = 133$) was found positive. Likewise, anti-IL-10 autoantibodies could not be found in the serum of patients suffering from myasthenia gravis, SLE, Crohn's disease, non-Hodgkin's lymphoma and malaria. However, four patients out of 300 suffering from bullous pemphigoid and three patients out of 400 suffering from chronic inflammatory arthritis presented IgG antibodies against hIL-10 in their sera (Table 1). As shown in Fig. 2, detectable autoantibodies to IL-10 were observed in sera from two positive patients (ANT and ODI) (suffering from bullous pemphigoid) down to a dilution of 1:16 and 1:32, respectively. The assay was specific, since the signal was completely blocked with a 100-fold molar excess of cold hIL-10 for all positive sera. An autoradiography of precipitates made with these positive anti-IL-10 autoantibodies shows essentially a 17-kD band which corresponds to the major component of the iodinated hIL-10 (the monomeric form of hIL-10) (data not shown).

For patient ANT, successive bleedings were carried out over a 17-month period and sera were tested for the presence of anti-IL-10 autoantibody. As shown in Fig. 3, anti-IL-10 autoantibodies could be detected at all time points, thus indicating the persistance of the B cell clone secreting the autoantibody.

Moreover, in five batches of human immunoglobulins for intravenous injections (IVIg), no anti-IL-10 autoantibodies were detected, whereas the five batches contained anti-IL-1α autoantibodies (data not shown).

Anti-IL-10 autoantibodies are specific

In order to rule out the possible detection of low-specificity antibodies (e.g. rheumatoid factors), the sera containing anti-IL-10 autoantibodies were tested for their reactivity with another cytokine, IL-1α. None of the sera samples did precipitate ^{125}I-IL-1α, while such anti-IL-1α autoantibodies are detected in about 10–20% of healthy subjects [20,21]. Also, preincubation of positive sera with IL-1α did not inhibit the

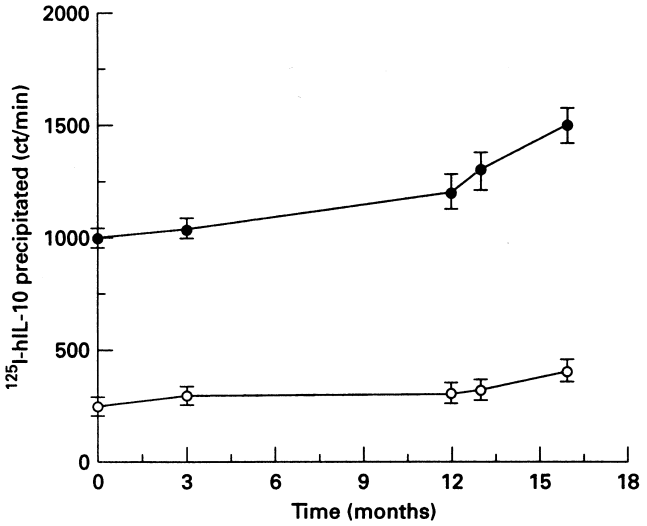

Fig. 3. Persistence of anti-IL-10 autoantibodies over 17 months. Sera samples from patient ANT were collected over 17 months and tested (at 1:4 dilution) using the immunoprecipitation technique with (○) or without (●) preincubation with a 100-fold molar excess of cold hIL-10.

formation of ^{125}I-hIL-10–anti-IL-10 autoantibody complexes. To further prove the specificity of the anti-IL-10 autoantibodies, these were purified on an affinity column composed of Affigel 10 to which highly purified human IL-10 was coupled. An affinity column composed of Affigel 10, to which highly purified human IL-4 was coupled, was used as control. The ammonium sulphate-precipitated and dissolved immunoglobulins of the positive serum ANT were loaded on the two columns, and effluents as well as eluates were subsequently tested for their capacity to immunoprecipitate ^{125}I-hIL-10. As observed in Fig. 4, after purification on hIL-10 Affigel 10 column, anti-hIL-10 activity was contained within the eluate but not in the flow-through. In contrast, after hIL-4 Affigel 10 column, anti-hIL-10 activity was present in the flow-through and not in the eluate, thus confirming the specificity of the assay.

To determine the isotype of the anti-IL-10 autoantibodies of one of the positive sera (ANT), immunoprecipitations were performed using anti-IgG1, IgG2, IgG3 or IgG4 heavy chains and anti-κ or -λ light chain antibodies coupled to Affigel 10 rather than Protein G-Sepharose. As shown in Table 2, the positive sera (ANT shown as an example) did not score better than the negative ones, probably because this assay is less sensitive than that based on Protein G-Sepharose. However, two independent eluates of hIL-10 Affigel 10 column from donor ANT were tested in these assays and only (IgG1, λ) antibodies could be detected. The precipitation was specific, since it was blocked by preincubation with a 100-fold molar excess of cold hIL-10. This result suggests the limited repertoire of the anti-IL-10 autoantibody in this particular patient.

Anti-IL-10 autoantibodies are directed against at least three different epitopes

To determine the fine specificity of the serum anti-hIL-10 autoantibodies, the seven positive sera were tested for their ability to bind vIL-10 and mIL-10. This was determined in a competition assay where sera were first preincubated with a 100-fold molar excess of either hIL-10, vIL-10 or mIL-10. The radioimmunoprecipitation procedure was performed by adding ^{125}I-hIL-10 and Protein G-Sepharose. The results illustrated in Fig. 5 demonstrate that four anti-IL-10 autoantibodies (JAY, HOD, BOU, BAJ) are specific for human IL-10, because neither vIL-10 nor mIL-10 can compete for the binding of hIL-10. Two anti-IL-10 autoantibodies were indeed able to recognize both human and viral IL-10, but were unable to bind to murine IL-10 (ANT, ODI). Finally, only one of the seven samples was able to recognize the three IL-10 (PAR). Note that there is no antibody that binds human and murine IL-10 but not vIL-10, a finding consistent with the degree of homology of those three forms of IL-10.

Taken together, these results indicate that at least three epitopes of hIL-10 are immunogenic and differentially recognized by human anti-IL-10 autoantibodies.

The anti-IL-10 autoantibodies inhibit IL-10 biological effects

The anti-IL-10 autoantibodies were assayed for their capacity to inhibit IL-10-induced proliferation of the mIL-3-dependent Ba/F3

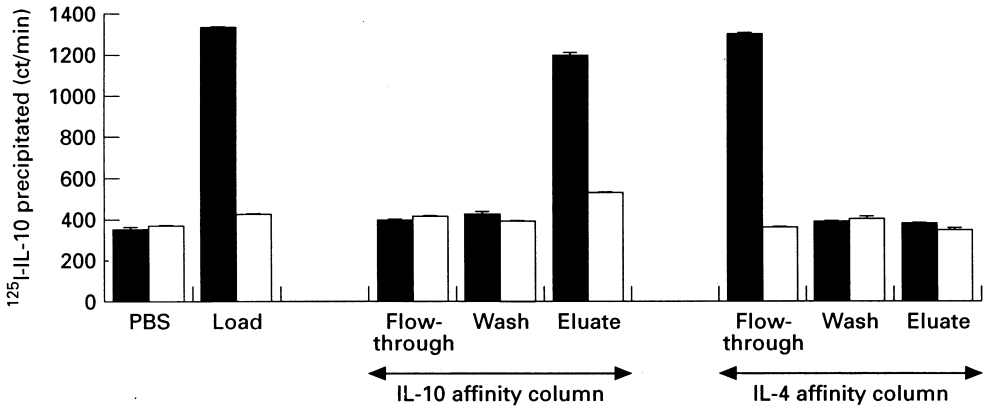

Fig. 4. Purification of human anti-IL-10 autoantibodies on hIL-10 and hIL-4 affinity columns. Ammonium sulphate-precipitated and dissolved immunoglobulins of ANT sera were added to 2 ml Affigel 10-hIL-10 or Affigel 10-hIL-4 affinity columns and after 12 h of incubation (4°C, rotation), flow-throughs were collected. After five PBS washes (5 × 2 ml), elution was performed with 0·1 M glycine buffer pH 2·7 and neutralized with Trizma base (0·1 M, pH 12). Loads, flow-throughs, washes and eluates of the two affinity columns were tested in the immunoprecipitation test after preincubation with medium (■) or a 100-fold molar excess of cold hIL-10 (□).

Table 2. Isotype of the anti-IL-10 autoantibody of patient ANT serum

ct/min		Anti-IgG1	Anti-IgG2	Anti-IgG3	Anti-IgG4	Anti-IgA	Anti-kappa	Anti-lambda
Negative serum	unprotected	311	364	397	458	171	222	235
	protected	317	358	342	415	233	200	225
ANT serum	unprotectd	246	435	496	424	254	320	279
	protected	254	475	365	487	255	340	297
Eluate exp. 1* (10.08.93)	unprotected	1431	490	563	502	833	1624	1472
	protected	558	395	437	528	820	1197	367
Eluate exp. 2* (18.08.93)	unprotected	1541	397	458	364	1573	ND	ND
	protected	479	420	372	297	1456	ND	ND

Ammonium sulphate-precipitated and dissolved immunoglobulins from ANT serum or hIL-10 affinity column eluate were preincubated with medium (unprotected) or a 100-fold molar excess of cold hIL-10 (protected) for 20 min and then incubated with ^{125}I-hIL-10 (20 000 ct/min) for 45 min. Immune complexes were immunoprecipitated with anti-IgG1, IgG2, IgG3, IgG4, IgA heavy chain antibodies or λ and κ light chain antibodies coupled to Sepharose. After incubation and washing, samples were counted on a γ counter. Each sample was tested in duplicate.

*Exp. 1 and exp. 2 were performed with ANT serum collected at 1 week intervals.

cells, that had been transfected with the human IL-10 receptor (hIL-10R$^+$ Ba/F3). As shown in Fig. 6, both hIL-10 and mIL-3 were able to induce hIL-10R$^+$ Ba/F3 cell proliferation as measured by a ^{3}H-TdR pulse after 48 h of culture. In pilot experiments, crude plasma and sera were demonstrated to give a proliferative signal to these cells, thus necessitating purification steps to assay the function of anti-IL-10 autoantibodies. The ammonium sulphate-precipitated and dissolved immunoglobulins from normal sera were found not to interfere with the spontaneous and mIL-3-dependent proliferation of hIL-10R$^+$ Ba/F3 cells (data not shown). In contrast, the ammonium sulphate precipitate of the ANT serum (that contain anti-IL-10 autoantibodies) inhibited by 50% the proliferation of hIL-10R$^+$ Ba/F3 cells induced by 2·5 ng/ml hIL-10. At 10 ng/ml hIL-10, the ammonium sulphate-enriched fraction inhibited the proliferation by 30%. In excess of hIL-10 (40 ng/ml), the ammonium sulphate-enriched fraction showed only a minor effect. The different fractions obtained on the hIL-10 affinity column were also tested for their ability to modulate IL-10-induced proliferation of hIL-10R$^+$ Ba/F3 cells. As shown in Fig. 6a, the hIL-10 column flow-through did not affect IL-10-induced proliferation. In contrast, the column eluate inhibited the hIL-10 biological effect. Note that none of the tested fractions affected mIL-3-induced proliferation of hIL-10R$^+$ Ba/F3 cells (Fig. 6b).

Thus, both the ammonium sulphate precipitate and the eluate of an hIL-10 affinity column obtained from ANT serum contained an anti-IL-10 autoantibody that was able to inhibit the activity of human IL-10.

DISCUSSION

The present study using a Protein G immunoprecipitation assay (which excludes low-affinity and low-specificity IgM natural antibodies) demonstrated the existence of IgG autoantibodies that are specific for human IL-10 in a small proportion of human sera. These autoantibodies are specific for IL-10 in that they

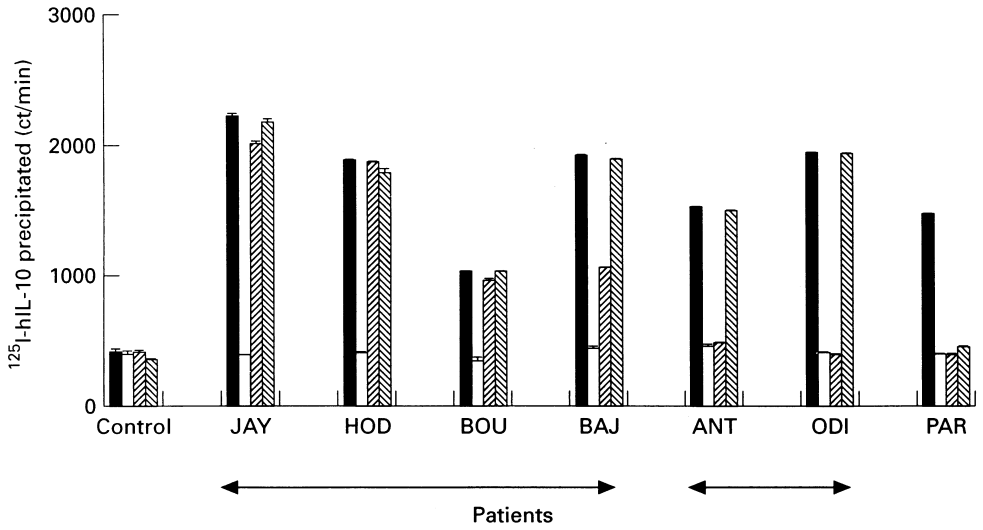

Fig. 5. Reactivity of anti-hIL-10 autoantibodies with viral and murine IL-10. Dilutions of positive sera were preincubated with either medium (■) or a 100-fold molar excess of cold hIL-10 (□), vIL-10 (▨) or mIL-10 (▧) for 20 min and then ^{125}I-hIL-10 was added in each well (20 000 ct/min) for 45 min. Immune complexes were immunoprecipitated with Protein G-Sepharose 4B and samples were counted. Each sample was tested in duplicate.

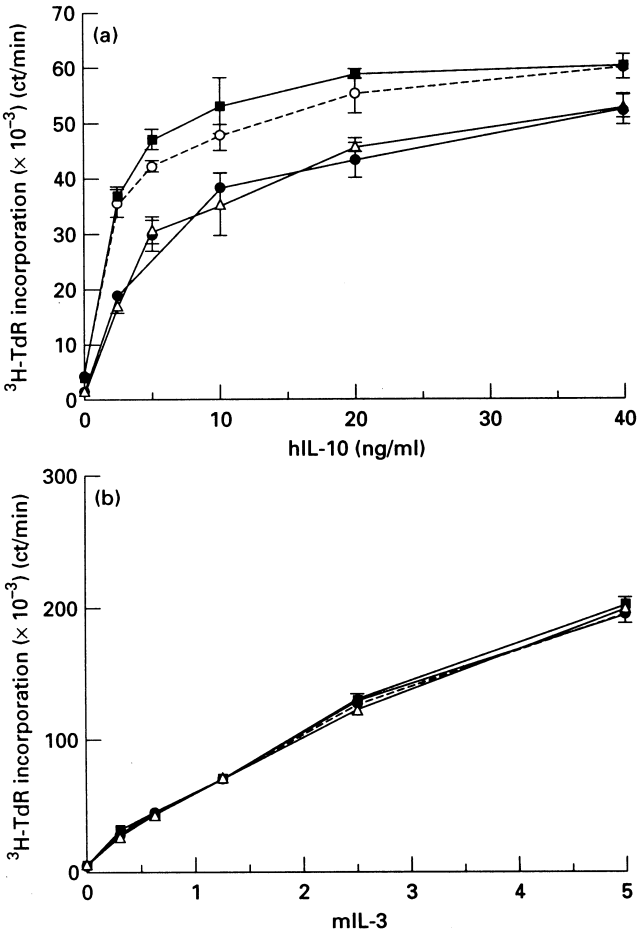

Fig. 6. ANT-purified anti-IL-10 autoantibody inhibits hIL-10- but not mIL-3-induced proliferation of hIL-10R$^+$ Ba/F3 cells. hIL-10R$^+$ Ba/F3 cells were washed three times in medium to remove mIL-3, and then 10^4 cells were distributed in 96-well round-bottomed tissue culture plates in 100 μl complete medium. Cells were incubated for 48 h with various concentrations of chinese hamster ovary (CHO)-purified hIL-10 (up to 40 ng/ml) (a) or various concentrations of mIL-3 (up to 5 ng/ml) (b). Cells were pulsed with ^{3}H-TdR for the last 18 h, harvested and counted. Tests were performed in triplicate and results expressed as mean ct/min ± s.d. Medium (○), ammonium sulphate-precipitated and dissolved immunoglobulins from ANT serum (12·5%) (●), hIL-10-Affigel 10 affinity column flow-through (10%) (■) and hIL10-Affigel 10 affinity column eluate (10%) (△) were tested in this proliferation assay.

recognized neither IL-1α in an immunoprecipitation assay which, in our hands, detects anti-IL-1α autoantibodies in 10–20% of sera (unpublished results, [20,21]), nor IL-4 as determined on an IL-4 affinity column. Analysis based on recombinant murine and viral IL-10 indicated the presence of at least three epitopic specificities on hIL-10. Four out of the seven autoantibodies to IL-10 were indeed *bona fide* autoantibodies, in that they recognized neither murine nor viral IL-10. However, three of these autoantibodies recognized both human and viral IL-10, therefore raising the question whether these autoantibodies may have in fact been originally directed towards vIL-10 rather than hIL-10. It will be of interest to determine whether antibodies specific to vIL-10, and not recognizing hIL-10, will be much more frequent than those specific to hIL-10, inasmuch as antibodies to Epstein–Barr virus antigens can be detected in a large proportion of healthy adults [22,23]. The frequency of anti-IL-10 autoantibody was low within the total population (0·57%). Also, the frequency of B cells producing such autoantibodies seemed low, as indicated by the oligoclonality found after analysis of purified antibodies from a serum that scored positive. In an independent study [16], up to three anti-IL-1α autoantibodies could be identified in a single individual. The low titres of anti-IL-10 autoantibodies in positive sera also indicated a limited clone size. The rare clone frequency and limited clone size of anti-IL-10 autoantibodies may indeed be the consequence of the fact that very few B cells can pass the positive and negative selection processes that are required to generate high-affinity autoantibodies [24,25]. On the other hand, the frequency of IL-10 autoantibodies may have been underestimated due to the widespread occurrence of IL-10, which is produced by numerous cell types, including T cells, B cells and monocytes, and which can be detected in ng/ml in serum of patients suffering from various pathologies, including lymphomas and malaria [26,27], therefore complexing the eventually circulating autoantibody. Note that the anti-IL-10-producing B cell clone appears to be long lived and to produce antibody continuously, as shown by the stable levels of anti-IL-10 autoantibody over a 17 month period. Such a property was earlier described with the anti-IL-1α autoantibodies [21].

Moreover, we tested, using our Protein G immunoprecipitation technique, five batches of human immunoglobulins for intravenous injections (IVIg) for the presence of anti-IL-10 autoantibodies. We could detect anti-IL-1α but not anti-IL-10 autoantibodies. This contrasts with previous reports [13,15] showing the presence of both anti-IL-1α and anti-IL-10 autoantibodies, a discrepancy which could be due to a difference in the sensitivity of the methods.

Due to the limited blood quantities, anti-IL-10 autoantibody was purified from only one serum (ANT). This autoantibody was able to neutralize the IL-10 biological activity, suggesting that such autoantibodies may represent antagonists to IL-10. Alternatively, these anti-IL-10 autoantibodies may increase IL-10 half life in serum, since anti-cytokine antibodies have been shown to increase the *in vivo* cytokine half life by preventing their proteolytic degradation [28–30]. While the seven anti-human IL-10 autoantibodies were detected in the serum of patients suffering from inflammatory disorders, including skin bullous diseases and chronic inflammatory arthritis, their low frequency does not allow us to decide at present whether their occurrence is indeed restricted to such disorders.

In conclusion, the present study has demonstrated the existence, in rare cases, of specific anti-human IL-10 neutralizing autoantibodies. It would be of interest to isolate the autoreactive B cell clones or the cDNA coding for such antibodies, as they could be used as IL-10 antagonists in diseases such as non-Hodgkin's lymphoma, AIDS-associated lymphomas and SLE, which have been reported to be associated with an excess of IL-10 [26,31,32].

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REFERENCES

1 Moore KW, O'Garra A, De Waal Malefyt R, Vieira P, Mosmann TR. Interleukin 10. Ann Rev Immunol 1993; **11**:165–90.

2 Mosmann TR. Cytokine patterns during the progression to AIDS. Science 1994; **265**:193–4.

3 Go NF, Castle BE, Barret R *et al.* Interleukin 10 (IL10), a novel B cell stimulatory factor: unresponsiveness of X-chromosome-linked immunodeficiency B cells. J Exp Med 1990; **172**:1625–31.

4 Rousset F, Garcia E, Defrance T *et al.* Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA 1992; **89**:1890–3.

5 de Waal Malefyt R, Abrams J, Bennett B, Figdor CG, de Vries JE. Interleukin 10 (IL-10) inhibits cytokine synthesis by human monocytes: an autoregulatory role of IL-10 produced by monocytes. J Exp Med 1991; **174**:1209–20.

6 Caux C, Massacrier C, Vanbervliet B, Barthelemy C, Liu YJ, Banchereau J. Interleukin 10 inhibits T cell alloreaction induced by human dendritic cells. Int Immunol 1994; **6**:1177–85.

7 Hsu H, De Waal Malefyt R, Fiorentino DF *et al.* Expression of interleukin 10 activity by Epstein–Barr virus protein BCRF1. Science 1990; **250**:830–2.

8 Vieira P, deWaal Malefyt R, Dang MN *et al.* Isolation and expression of human cytokine synthesis inhibitory factor cDNA clones: homology to Epstein–Barr virus open reading frame BCRFI. Proc Natl Acad Sci USA 1991; **88**:1172–6.

9 Chomarat P, Rissoan MC, Banchereau J, Miossec P. Interferon γ inhibits interleukin 10 production by monocytes. J Exp Med 1993; **177**:523–7.

10 Ross C, Hansen MB, Schyberg T, Berg K. Autoantibodies to crude human leucocyte interferon (IFN), native human IFN, recombinant human IFNα2b and human IFNγ in healthy blood donors. Clin Exp Immunol 1990; **82**:57–62.

11 Hansen MB, Svenson M, Diamant M, Bendtzen K. High affinity IgG autoantibodies to IL6 in sera of normal individuals are competitive inhibitors of IL6 *in vitro*. Cytokine 1993; **5**:72–80.

12 Svenson M, Poulsen LK, Fomsgaard A, Bendtzen K. IgG autoantibodies against interleukin-1 alpha in sera of normal individuals. Scand J Immunol 1989; **29**:489–92.

13 Bendtzen K, Hansen MB, Diamant M, Ross C, Svenson M. Naturally occurring autoantibodies to interleukin 1α, interleukin 6, interleukin 10, and interferon-α. J Interferon Res 1994; **14**:157–8.

14 Monti E, Pozzi A, Tiberio L *et al.* Purification of interleukin-2 antibodies from healthy individuals. Immunol Letters 1993; **36**:261–6.

15 Bendtzen K. Cytokines and natural regulators of cytokines. Immunol Letters 1994; **43**:111–23.

16 Garronne P, Djossou O, Fassieg F *et al.* Generation and characterization of a human monoclonal autoantibody that acts as a high affinity interleukin-1 alpha specific inhibitor. Mol Immunol 1996; in press.

17 Liu Y, Wei Y, Ho AS, De Waal Malefyt R, Moore KW. Expression, cloning and characterization of a human IL10 receptor. J Immunol 1994; **152**:1821–9.

18 Ho AS, Liu Y, Khan T, Hsu DH, Bazan JF, Moore KW. A receptor for interleukin 10 is related to interferon receptors. Proc Natl Acad Sci USA 1993; **920**:11267–71.

19 Abrams JS, Roncarolo MG, Yssel H, Andersson U, Gleich GJ, Silver JE. Strategies of anticytokine monoclonal antibody development: immunoassay of IL10 and IL5 in clinical samples. Immunol Rev 1992; **127**:5–24.

20 Svenson M, Hansen MB, Bendtzen K. Binding of cytokines to pharmaceutically prepared immunoglobulins. J Clin Invest 1993; **92**:2533–9.

21 Suzuki H, Ayabe T, Kamimura J, Hashwiwagi H. Anti IL1α autoantibodies in patients with rheumatic diseases and in healthy subjects. Clin Exp Immunol 1991; **85**:407–12.

22 Ritter K, Kuhl RH, Semrau F, Eiffert H, Kratzin HD, Thomssen R. Manganese superoxide dismutase as a target of autoantibodies in acute Epstein–Barr virus infection. J Exp Med 1994; **180**:1995–8.

23 Marchini B, Dolcher MP, Sabbatini A, Klein G, Migliorini P. Immune response to different sequences of the EBNA-1 molecule in Epstein–Barr virus related disorders and in autoimmune diseases. J Autoimmun 1994; **7**:179–91.

24 MacLennan ICM. Germinal centers. Ann Rev Immunol 1994; **12**:117–39.

25 Nossal GJV. Negative selection of lymphocytes. Cell 1994; **76**:229–39.

26 Blay JY, Burdin N, Rousset F *et al.* Pronostic value of serum IL10 in non Hodgkin's lymphoma. Blood 1993; **82**:2169–74.

27 Peyron F, Burdin N, Ringwald P, Vuillez JP, Rousset F, Banchereau J. High levels of circulating IL-10 in human malaria. Clin Exp Immunol 1994; **95**:300–3.

28 Bendtzen K, Svenson M, Jonsson V, Hippe E. Autoantibodies to cytokines—friends or foes? Immunol Today 1990; **11**:167–9.

29 Finkelman FD, Madden KB, Morris SC *et al.* Anticytokine antibodies as carrier proteins. Prolongation of *in vivo* effects of exogenous cytokines by injection of cytokine–anticytokine antibody complexes. J Immunol 1993; **151**:1235–44.

30 Suzuki H, Takemura H, Yoshizaki K *et al.* IL6–anti IL6 autoantibody complexes with IL6 activity in sera from some patients with systemic sclerosis. J Immunol 1994; **152**:935–42.

31 Emilie D, Touitou R, Raphael N *et al. In vivo* production of interleukin-10 by malignant cells in AIDS lymphoma. Eur J Immunol 1992; **22**:2937–42.

32 Llorente L, Zou W, Levy Y *et al.* Role of interleukin 10 in the B lymphocyte hyperactivity and autoantibody production of human systemic lupus erythematosus. J Exp Med 1995; **181**:839–4.