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Monitoring of interleukin-2 receptor (IL-2R) expression *in vivo* and studies on an IL-2R-directed immunosuppressive therapy of active and adoptive adjuvant-induced arthritis in rats

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SUMMARY

Recent evidence indicates that adjuvant arthritis (AA) of rats induced by complete Freund's adjuvant (CFA) is an autoimmune disease that is mediated by T cells. This report describes the distribution of activated IL-2 receptor (IL-2R)-bearing cells in spleen, popliteal lymph nodes (PLN) and blood in AA rats and in naive healthy rats using the monoclonal antibody (mAb) ART-18. It was found that in the primary lymph nodes (injected side) two peaks of elevated numbers of IL-2R-positive cells (Day 9/10 with a 40-fold increase; Day 25 with a 75–80-fold increase) occur. The PLN of the non-injected site also show an increase (30-fold) in the number of IL-2R-positive cells on Day 25. This investigation also included the monitoring of soluble IL-2R in the serum of AA rats in comparison to control sera of non-induced rats. The incidence of free IL-2R in the serum of AA rats does not completely correlate with the pattern of the distribution of receptor-bearing cells in PLN; elevated levels of IL-2R were observed at Day 9 and subsequently declined to below control levels. On Day 25, there was no correlation between IL-2R$^+$ cells and soluble IL-2R. ART-18 was not active in suppressing the development of AA, in contrast to the complete inhibition of the passively transferred AA.

INTRODUCTION

Adjuvant arthritis (AA) in rats, induced by injection of complete Freund's adjuvant (CFA), is characterized by the development of a polyarthritis with subsequent irreversible ankylosis of the joints (Stoerk, Bielinski & Budzilovich, 1954; Pearson, 1956). The disease is thought to be based on cellular T-cell-mediated immunological mechanisms (Kayashima, Koga & Onoue, 1976; Iizuka & Chang, 1982). Recent studies using arthritogenic T-cell lines support the postulation that the AA—for which the exact antigen is still unknown—is an autoimmune phenomenon, based on cross-reactivity between the antigenic determinants of the mycobacteria and those of the joints (Holoshitz, Matitiau & Cohen, 1984). Van Eden *et al.* (1985) have shown that the arthritogenic T-cell line A2b recognizes parts of the proteoglycan molecules of the cartilage. Therefore it is of great interest to investigate the activation of T cells *in vivo* or *ex vivo*. Antigen-activated T cells are characterized by the expression of IL-2 receptors (Robb, Munck & Smith, 1981; Smith *et al.*, 1979). Because the expression of IL-2R is a specific event that allows the differentiation of antigen-activated T cells and resting T cells, it should be possible to detect activated T cells *in vivo* or *ex vivo* using IL-2R-specific ligands (Volk *et al.*, 1986). Taking this into account, the recently developed IL-2R-specific monoclonal antibody (mAb) ART-18, which inhibits the binding of IL-2 to its receptor and the subsequent proliferation in response to IL-2, was chosen for such an approach.

Another aspect of using this mAb is the immunotherapy proposed by Diamantstein & Osawa (1986). Conventional therapy using immunosuppressive drugs often results in a high level of unwanted side-effects. The development and use of mAb raised against T cells or T-cell subsets abolishes some of the problems of general immunosuppression. Our concept of immunosuppression is based on specific elimination of activated T cells responsible for the unwanted immune reaction. In this context ART-18 could be successfully administered to different animal models with T-cell-dependent immune reactions such as graft-versus-host (GvH) reaction and host-versus-graft (HvG) reaction in organ transplantations (Volk *et al.*, 1986; Kupiec-Weglinski *et al.*, 1988).

In this study, the *in vivo* activation of rat T cells during the course of AA was examined by determination of IL-2R-bearing cells in spleen, peripheral blood and popliteal lymph nodes and

Abbreviations: AA, adjuvant arthritis; BSS, balanced salt solution; CFA, complete Freund's adjuvant; ELISA, enzyme-linked immunosorbent assay; IL-2R, interleukin-2 receptor; mAb, monoclonal antibody; PLN, popliteal lymph node.

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of the levels of soluble IL-2 receptors in the sera of the rats. Furthermore, efforts have been made to suppress the development of adjuvant arthritis by ART-18 immunotherapy.

MATERIALS AND METHODS

Animals

Inbred female Lewis rats of 120–150 g body weight were purchased from Moellegard Breeding Center, Ejby, Denmark. Animals were kept under standard conditions on a standard diet and water *ad libitum*.

Monoclonal antibodies

Unless otherwise stated, the monoclonal antibodies (mAb) ART-18 and ART-65 (Mouzaki *et al.*, 1987b) (mouse mAbs of IgG1 isotype recognizing the IL-2 receptor on activated T cells) were used in a purified form. Production and purification of ascites fluids were done by ammonium sulphate precipitation and subsequent ion-exchange chromatography (Kupiec-Weglinski *et al.*, 1988) or by successive binding/elution from protein A–Sepharose (Pharmacia, Freiburg) as previously described (Ey, Prowse & Jenkin, 1978). Purified ART-65-IgG1 was biotinylated as reported by Osawa, Josimovic-Alasevic & Diamantstein (1986). Purified mouse myeloma protein was used as control (MOPC 21, Litton Bionetics Inc., Charleston, SC).

Cell preparations and culture conditions

Spleens and lymph nodes were removed from the killed animals under sterile conditions and teased with forceps in ice-cold BSS. After brief sedimentation to remove coarse particles, the cell suspension was washed twice in 40 ml of BSS and subsequently resuspended in culture medium RPMI-1640 (Flow Laboratories, Meckenheim) containing 25 U/ml penicillin, 25 μg/ml streptomycin, 200 mM L-glutamine and 5% FCS (Gibco/BRL Eggenstein). Contaminating erythrocytes were depleted by use of Tris-ammonium chloride solution. Cell counts and viability testing by the dye exclusion method (0·5% trypan blue) were performed in a Neubauer haemocytometer.

Heparinized blood was obtained by cardiac puncture of previously killed animals. Mononuclear cells were separated by density gradient centrifugation (Lympho–paque. Nyegaard, Oslo, Norway; density 1·086 g/ml) and then washed three times with BSS.

Induction and evaluation of adjuvant arthritis (AA)

Heat-killed *Mycobacterium butyricum* (Difco, Detroit, MI) was suspended in paraffin oil (5 mg/ml, Merck, Darmstadt (CFA) and injected into the footpad (50 μl) of the right hind paw of the Lewis rats. Rats of the same weight were injected with 50 μl of paraffin oil and served as controls. In order to evaluate the progression of the disease two parameters were defined: (i) the swelling of both hind paws using a micro-amperemeter (Kemper & Ameln, 1958); (ii) the arthritic score, involving the quantification of seven arthritic signs on the three non-injected paws, ears, nose and tail. Parameters of the evaluation were swelling, edema and erythema of the involved sites. The polyarthritis was assessed every third day for 60 days post-injection.

Passive transfer of adjuvant arthritis

The passive transfer of AA was performed according to the method of Taurog, Sandberg & Mahowald (1983). In brief, spleen cells of donor rats, obtained on Day 11 of disease, were cultured in the culture medium described, with addition of 2-mercaptoethanol (5×10^{-5}M) and Concanavalin A (Con A, 4 μg/ml, Pharmacia Fine Chemicals, Uppsala, Sweden) at 37°. After 72 hr, cells were harvested and 1×10^8 cells, suspended in BSS, were injected into the tail vein of naive recipients. The disease was evaluated as described above. The score in this model includes eight arthritis signs, because both hind paws are included in the estimation.

Determination of lymph node weights and mononuclear cell counts

Popliteal lymph nodes of CFA-injected and non-injected sites were removed at different times after the beginning of the study and weighed (Sauter balance, Ebingen). Cell counts of the nodes were determined subsequently.

Immunofluorescence

Immunofluorescence was performed as previously described (Munker *et al.*, 1983). Briefly, 1×10^5 isolated cells of popliteal lymph nodes (PLN), spleen and peripheral blood were suspended in staining buffer (Dulbecco-PBS, Gibco/BRL, 0·3% bovine serum albumin, 0·1% NaN_3) and were incubated with 50 μl ascites fluid of the monoclonal antibody ART-18 (1:50 dilution) or of an irrelevant mouse myeloma protein of the same isotype (1:50 dilution, MOPC 21, clarified ascites, Litton Bionetics Inc., Charleston, SC) for 30 min at 4°. After three washing steps, 50 μl of a rat anti-mouse fluorescein-conjugated antibody (Jackson ImmunoResearch Labs, Avondale, PA) were added (1:20, 30 min at 4°). Fluorescence was analysed on the fluorescence-activated cell sorter (FACSII, Becton-Dickinson, Mountain View, CA) according to standard methods (Loken & Herzenberg, 1975).

ELISA for soluble IL-2R

The ELISA was performed as described recently (Mouzaki, Osawa & Diamantstein, 1987a). In brief, 0·1 ml of purified ART-18-IgG1 (10 μg/ml) in PBS containing 0·02% NaN_3 (PBS/NaN_3) were incubated at 4° for 48 hr in flexible 96-well polyvinyl chloride microtitre plates (Flow Laboratories). After washing procedures (PBS/0·1% Tween) and saturation to block non-specific binding with solutions containing 3% BSA, 0·1 ml of two-fold serial dilutions of the samples containing the rat IL-2R and control samples (IL-2R negative) were added. After incubation for 1 hr at 37° and further washings, reaction with 0·1 ml of 1:50 dilution, biotinylated ART-65IgG1 (260 μg/ml) in PBS/NaN_3 containing 1% BSA for 1 hr at 37° followed. Bound ART-65 was detected by incubation (1 hr, 37°) with 0·1 ml of a 1:1000 dilution of streptavidin-alkaline phosphatase conjugate for 10 min at room temperature. Subsequently, the unbound conjugates were removed by washing. For the enzymatic reaction 0·1 ml of alkaline phosphatase substrate containing 0·1 mg/ml of *p*-nitro-phenyl/phosphate, disodium (Sigma Chemie, München), 1 M diethanolamine-HCL (pH 9·8), 0·24 mM $MgCl_2$ at 37° for 30 min was used. The reaction, stopped by addition of 0·6 M NaOH, was determined at 405 nm in a Titertek ELISA reader (Flow Laboratories).

Solubilized rat T-lymphoblast extracts derived from 10^8 cells/ml were arbitrarily assigned a concentration of 1000

Table 1. Quantification of the proportion of IL-2R-bearing cells of various lymphoid tissues before and after stimulation

Tissue	Lymphocytes (% IF-positive cells)		
	Non-activated	Activated	
		Mitogen	Alloantigen
Naive rats			
Spleen	2·1	90	37·5
LN	3·1	81	ND
Blood	1·5	ND	ND
AA rats			
Spleen	2·2	82	ND
LN	3·1	91	ND
Blood	1·8	ND	ND

Indirect immunofluorescence (IF) analysis was done with mouse anti-IL-2R mAb ART-18 and a rat anti-mouse IgG-FITC-labelled antiserum on cells of various tissues (spleen, lymph nodes (LN), blood) of naive and CFA-induced arthritic rats (15 days after CFA injection). In addition, aliquots of cells were cultured under stimulatory conditions [Con A (mitogen) for 3 days; alloantigen (MLR; Lewis versus Wistar) for 5 days] before IF analysis was carried out. The values are means of three animals from two different series of experiments.

ND, not done.

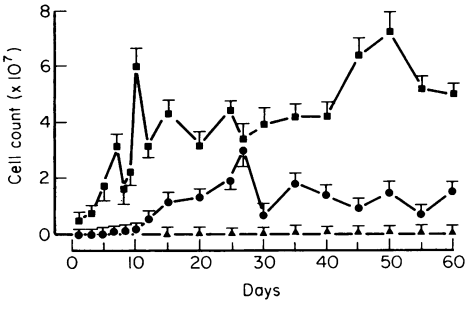

Figure 1. Cell counts of the primary (injected site) (■) and secondary (non-injected site) (●) PLN in the course of AA in comparison to PLN of naïve non-CFA-injected animals (▲); means of triplicate experiments ± SEM; $P < 0{\cdot}001$.

soluble IL-2R U/ml and were used as internal standards. The cell extracts were produced by lysing Concanavalin A (Con A)-stimulated T lymphoblasts at a concentration of 10^8/ml in PBS which contained 1% w/v Triton X-100 and 2 mM phenylmethylsulphonyl fluoride (PBS/TX-100) as described elsewhere (Osawa & Diamantstein, 1984).

ART-18 mAb treatment

Recipients (five to seven per group) were injected with 1 mg purified mAb diluted in physiological saline/kg body weight/day, intraperitoneally, according to various administration schemes. The effect of therapy upon the progression of the disease was monitored as described above.

Statistics

The results were analysed for their statistical significance using the Student's *t*-test and the Mann–Whitney *U*-test.

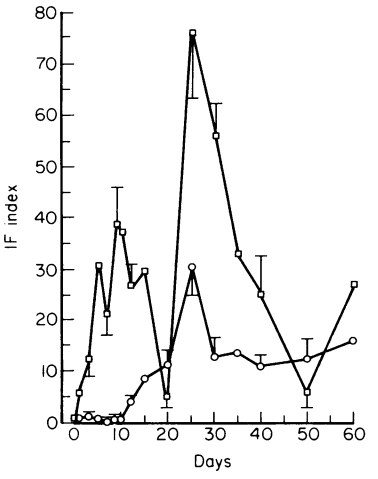

Figure 2. Determination of ART-18-positive cells in primary (injected site) (□) and secondary (non-injected site) (○) popliteal lymph nodes during the course of AA; 1×10^6 cells were incubated with ART-18 (1:50 dilution) and subsequently with FITC-conjugated polyclonal rat anti-mouse IgG antibodies (1:20 dilution) according to the indirect immunofluorescence method. The immunofluorescence index (IF index) represents the ratio of absolute cell counts of ART-18$^+$ cells in AA rats to those of naïve controls (means of three experiments).

RESULTS

Occurrence of IL-2 receptor-bearing cells during AA

IL-2R is expressed in naive untreated rats in spleen, blood and popliteal lymph nodes only by a minor population of cells ($\geq 3{\cdot}5\%$). Stimulation of isolated cells of PLN and spleen with Con A as a control revealed that more than 80% of cells of spleen and PLN bear the IL-2 receptor (Table 1).

As judged by fluorescence-activated cell sorter analysis, kinetic studies on the IL-2R-expression of cells of peripheral blood, spleen and PLN in AA rats showed the same frequency of ART-18-positive cells as the rats of the control group ($\geq 3{\cdot}5\%$). This level of IL-2R$^+$ cells remained constant for the whole period of observation (60 days). In parallel to the fluorescence analysis the cell counts in the different organs were estimated and showed that, in contrast to spleen and peripheral blood, cell counts in both PLN are greatly elevated during the course of the disease (Fig. 1). Comparing the nodes from the injected site with those of the non-injected site differences could be seen between the level and onset of the elevated number of cells. The cell count of the node of the injected site (primary site) increases continuously up to Day 10 (approximately 120-fold increase, about 6×10^7 cells; level of PLN of naive rats 5×10^5). The increase of the other nodes (secondary site) is less pronounced (approximately 30-fold increase) with a delayed onset at Day 10. This day is considered to be the start of the systemic disease. Thus a striking difference in the number of ART-18-positive cells is evident between spleen and peripheral blood on the one hand and PLN on the other. The number of IL-2R-bearing cells in the nodes of the primary site shows two peaks, a first on Days 9–10 and a second on Day 25, which is paralleled by the secondary PLN. In this case the beginning of the rise of fluorescent cells is identical to the beginning of the enhancement of the absolute cell number (Fig. 2). These observations suggest that in AA rats a pro-

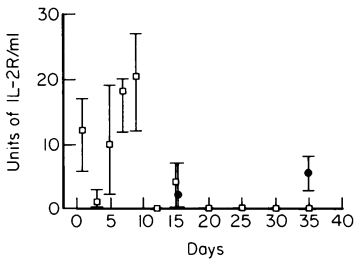

Figure 3. Detection of soluble IL-2R in the sera of AA rats (open symbols) and normal controls (closed symbols); means of triplicate ±SEM.

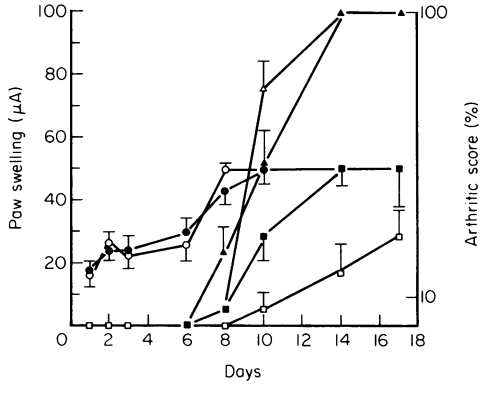

Figure 4. ART-18 therapy of active AA; rats were treated i.p. with 1 mg/kg body weight/day (Days 0–6; closed symbols); the control group was untreated (open symbols); evaluation was made by measurement of the injected (circle) and non-injected (square) paw (μA) and by quantification of the score (triangle) (%).

nounced proliferation and accumulation of activated cells occurs in PLN as the draining lymph nodes of one of the sites of arthritis, in contrast to spleen and blood, and that some of them bear IL-2R.

Detection of soluble IL-2R in rat sera

On Days 0–35, sera of AA rats were obtained and tested for IL-2R levels by the ELISA. On Days 0, 15 and 35 naive rats served as controls, since it has been shown that IL-2R are also detectable in healthy animals (Mouzaki *et al.*, 1987 2a). Figure 3 shows the results as mean values of IL-2R U/ml per group. The results demonstrate that high IL-2R levels can be detected in the sera during the first 10 days of the course of the disease, reaching a peak on Day 9 (>20 units of IL-2R/ml) and then sharply declining to below control serum IL-2R levels and, finally, reaching ELISA background levels on Day 20 and thereafter.

Therapeutic use of ART-18 in active and passive AA

As shown in Fig. 4, administration of ART-18 (1 mg/kg, Days 0–6 intraperitoneal) had no effect on the course of the active AA. Neither the swelling of the hind paws nor the arthritic score showed any improvement compaired to the untreated control. Different therapeutic schedules (Days 0–10; 0–2/7–9; 5–10; 5–7) and different doses (300 μg–5000 μg/kg) were equally ineffective in the active AA.

In contrast, using the model of the adoptive transfer of AA, the development of the disease could be inhibited completely by ART-18 given at Days 0–6 or 0–10 (Table 2, Fig. 5). Controls receiving myeloma protein of the appropriate isotype leaves the AA in the recipients unaffected.

DISCUSSION

The aim of the present study was to examine the distribution of activated T cells during the course of AA in rats and to determine the kinetics of soluble IL-2R in the serum. Furthermore, the attempt was made to influence the disease by IL-2R-targeted mAb therapy.

AA of rats as a T-cell mediated autoimmune disorder is characterized by two phases of the disease, an acute phase from the day of CFA-injection until Day 9/10 and a systemic phase starting at Day 9/10 with the expression of arthritic signs on the whole body. During the search for parameters to evaluate the AA others than paw-swelling and score we found out that the cell counts of the popliteal lymph nodes show a characteristic development. The cell count of primary PLN of the injected site is greatly increased up to Day 10 (120-fold increase) and subsequently stays at an elevated level (80–120-fold increase). The expansion of the secondary PLN of the non-injected site starts at the same time when paw swelling becomes evident, reaching a 40-fold enhancement at Day 25. The cell counts of spleen and peripheral blood, however, remain unaltered during the course of the disease. Panosian (1984) first demonstrated that, in AA, the T-cell population in the PLN is increased due to an enhancement of the T-helper subset and not of T-suppressor cells. Our results indicate that the enlargement of the PLN, expressed by the elevated cell counts, is accompanied by an increase in absolute numbers of IL-2R-bearing cells. The IL-2R-positive cells in the primary PLN show two peaks, one at Day 9/10 with a 40-fold increase compared to the non-treated control, and a second peak with a 75–80-fold increase. The secondary PLN shows a delayed onset of swelling on Day 11, the stage of systemization. In this case, the number of IL-2R-bearing cells reaches the level of a 30-fold increase on Day 25. The reason for the elevated levels of IL-2R-expressing cells at the very late stages of disease, for example on Day 60, is not known. A possible explanation is the recogniton of autoantigens, according to the findings of Holoshitz *et al.* (1983) who established arthritogenic T-cell lines of the helper phenotype out of PLN of AA rats. Van Eden *et al.* (1985) demonstrated that these cell lines recognize parts of the proteoglycan molecule of the cartilage due to structural mimicry; an alternative hypothesis is the continuous release of antigen by the oily suspension of CFA, which serves as a depot and/or by continuous release of cell wall components of phagocytized and only partially degraded *M. butyricum* by macrophages as reported for streptococci (Ginsburg, Zor & Floman, 1977).

The expression of IL-2R is a transient step in the common pathway of T-cell activation (Smith *et al.*, 1979; Coutinho *et al.*, 1979). The soluble receptor can be determined in the supernatant of activated T cells as well as in the serum of mice and rats (Rubin, Jay & Nelson, 1986; Osawa *et al.*, 1986; Mouzaki *et al.*, 1987a). The incidence of cell-free IL-2R in the serum of AA rats does not completely fit into the occurrence of receptor-bearing cells in the PLN. There is an elevated level of IL-2R at Day 9 with a subsequent sharp decline to below control levels.

Table 2. Arthritic signs during the course of adoptive adjuvant arthritis in rats treated with mAb ART-18 and their appropriate controls

Day post-transfer	Therapy ART-18 (Days 0–6)	Arthritic signs (no. positive animals)								Score‡ (%)
		FP_R*	FP_L†	FP_R	FP_L	Ear_R	Ear_L	Nose	Tail	
		Back		Front						
0–4	–	0/7	0/7	0/7	0/7	0/7	0/7	0/7	0/7	0
	+	0/7	0/7	0/7	0/7	0/7	0/7	0/7	0/7	0
5	–	3/7	1/7	0/7	0/7	1/7	0/7	4/7	1/7	19,6
	+	0/7	1/7	0/7	0/7	0/7	0/7	2/7	0/7	5,4
6	–	3/7	3/7	0/7	2/7	1/7	0/7	6/7	1/7	28,6
	+	0/7	1/7	0/7	0/7	0/7	0/7	1/7	0/7	3,6
7	–	4/7	4/7	0/7	2/7	1/7	0/7	6/7	3/7	35,7
	+	0/7	1/7	0/7	0/7	0/7	0/7	0/7	0/7	1,8
10	–	4/7	4/7	0/7	2/7	1/7	0/7	6/7	4/7	37,5
	+	0/7	1/7	0/7	0/7	0/7	0/7	0/7	0/7	1,8
12	–	5/7	3/7	0/7	2/7	1/7	0/7	6/7	3/7	35,7
	+	0/7	1/7	0/7	0/7	0/7	0/7	0/7	0/7	1,8
14	–	6/7	3/7	0/7	2/7	1/7	0/7	6/7	3/7	37,5
	+	0/7	1/7	0/7	0/7	0/7	0/7	0/7	1/7	3,6
17	–	6/7	4/7	0/7	2/7	1/7	0/7	4/7	3/7	35,7
	+	0/7	1/7	0/7	0/7	0/7	0/7	0/7	1/7	3,6
19	–	6/7	3/7	0/7	2/7	1/7	0/7	4/7	2/7	32,1
	+	0/7	1/7	0/7	0/7	0/7	0/7	0/7	1/7	3,6

*FP_R, right foot pad.
†FP_L, left foot pad.
‡Sum of arthritic signs expressed as percentage.

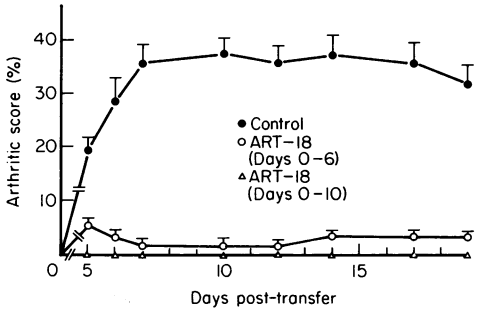

Figure 5. Therapeutic use of mAb ART-18 in the passively transferred AA. For the passive transfer of the disease, spleens of AA rats were removed on Day 11, suspended and stimulated *in vitro* with the mitogen Con A for a period of 3 days and subsequently injected i.v. (1×10^8). Rats were treated i.p. with 1 mg ART-18/kg body weight/day. Values are expressed as means ($n = 7$) ± SEM.

Unexpectedly, the peak of activated T cells at Day 25 was not seen here. The function of the soluble IL-2R is still unknown. Osawa *et al.* (1986) and Rubin *et al.* (1986) demonstrated that the released IL-2R binds to purified recombinant IL-2. For this interaction, no additional accessory molecules seem to be necessary. The ability of the receptor in its solubilized form to bind IL-2 suggests a possible regulation of cell activation by the released receptor subunit. It is possible that a down-regulation of cell activation is achieved by absorbing IL-2 to the soluble receptor. In this respect, an inhibition of the *in vitro* proliferation of T cells was observed after addition of the released IL-2R (Rubin *et al.*, 1986). Under *in vivo* conditions this may have the means to reduce the availability of IL-2 and therefore restrict the immune response to a certain locality. The trigger for the receptor release is unclear. It was shown by Ziai *et al.* (1985) that IFN-γ caused an increase in the cell surface expression and in the shedding of HLA class II antigens from IFN-γ-resistant subcloned melanoma cells. Similar results were reported by Khayat *et al.* (1984) who found soluble Fc receptors for IgG in the serum of mice. Based on observations with germ-free mice, it was suggested that the release of the Fc-IgG receptors is correlated with the activation of the immune system. Further evidence for this thesis was given by Pure *et al.* (1984) who found that the amount of soluble FcγR was increased dramatically by stimulation with lipopolysaccharide.

An ideal treatment of an autoimmune disorder should eliminate the autoreactive clones while sparing the other immunocompetent cells. Since the autoantigen is not known in AA an approach would be to concentrate on the specifically activated T cells which express IL-2R during the proliferative burst. Therefore an IL-2R-targeted therapy by the use of the mAb ART-18 may eliminate the undesired immune response.

Two different results using ART-18 in the model of AA are striking; it has been impossible to influence the active AA by different application schedules. In contrast, the passively transferred AA could be completely inhibited. The active AA has been shown to be delayed by injection of W 3/13 (anti-pan T-cell antibodies), but not by application of OX8 mAb (anti-suppressor/cytotoxic T-cell antibodies) (Larsson *et al.*, 1985). A

complete inhibition was not achieved. The reason for the failure of the ART-18 therapy of active AA is not clear. It is possible that the antigenic stimulus, continuously released by the oily suspension of CFA, is too strong to be overcome and that the activated T cells are not accessible to ART-18 because of their distribution in the organism, e.g. in the PLN and or in the synovium (Larsson *et al.*, 1985). However, studies of different models well known to be T-cell-mediated, like GvHR and DTH, demonstrate that anti-IL-2R targeted therapy inhibits the immune reactions in the recipient animal (Volk *et al.*, 1986; Kelley *et al.*, 1986). Therefore, the possibility cannot be ruled out that T cells are not solely responsible for active AA. As shown recently by Herrmann *et al.* (1985) IFN-γ-activated monocytes possess receptors for IL-2 as well. Analysis of tissue sections of cardiac allografted rats revealed a significant proportion of IL-2R-expressing macrophages infiltrating the rejected tissue (Hancock *et al.*, 1987). Thus, those cells may already absorb a substantial amount of the injected anti-IL-2R mAb at the inflammatory site or in the draining lymphatic tissue containing immunoreactive cells.

In the passively transferred AA, however, activated T cells are injected intravenously and can probably be inactivated or eliminated by ART-18 before they reach the inflammatory sites. These results are similar to those of Weckerle & Diamantstein (1986) who inhibited the development of passively transferred experimental autoimmune encephalomyelitis (EAE) by only one injection of ART-18.

The limited immunotherapeutic success with the monoclonal anti-IL-2R antibody might therefore be a problem of the locally achievable antibody concentration in such an antigenically strong T-cell-mediated inflammatory model. For a successful treatment of active AA further investigations into the pathogenesis of the disease as well as the role of macrophages have to be carried out.

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