Immunology 1997 **91** 260–265

Polyclonal Th1 cells transfer oil-induced arthritis

L. SVELANDER,* Å. MÜSSENER,* H. ERLANDSSON-HARRIS* & S. KLEINAU*† **Department of Rheumatology, Karolinska Hospital, Stockholm and †Department of Pathology, University Hospital, Uppsala, Sweden*

SUMMARY

T-cells play a critical role in oil-induced arthritis (OIA) in DA rats. The present study focuses on the involvement of CD4/CD8 T cells in OIA by using adoptive transfer. Mitogen-activated T cells from DA rats previously injected with incomplete Freund's adjuvant (IFA) were depleted of $CD4^+$ T cells or $CD8^+$ T cells before transfer to irradiated naive receipients. The results indicate that $CD4^+$ T cells are essential for the induction of passively induced OIA. However, *in vitro* blocking experiments with monoclonal antibodies (mAb) to the CD4 molecule of the T cells before transfer did not affect the passive OIA. Neither was passive OIA inhibited by treating the $CD4^+$ T cells with mAb to intracellular adhesion molecule-1 (ICAM-1) in order to block cell–cell interactions or migration. The arthritogenic $CD4^+$ T cells were sensitive, however, to *in vitro* treatment with mAb to the interleukin-2 receptor, which inhibited the disease or delayed the onset of passive OIA in recipients. The arthritogenic $CD4^+$ T cells were also analysed for expression of specific T-cell receptor (TCR) variable (V)β chains, critical for recognition of autoantigen, by utilizing Vβ gene-specific polymerase chain reaction (PCR). The results show a heterogeneous expression of Vβ segments of the TCR, indicating a polyclonal origin of the pathogenic cells. Moreover, an investigation of the T helper (Th)1/Th2 status of the $CD4^+$ T cells, defined by cytokine expression, was made at the mRNA level by using *in situ* hybridization. High numbers of interleukin-2 (IL-2) mRNA expressing cells and also interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α)-expressing cells could be identified. We conclude from this study that non-immunogenic IFA triggers polyclonal, IL-2-dependent Th1 cells which induce arthritis. The contribution of the CD4 or ICAM-1 molecules for arthritis induction seem to be of minor importance.

INTRODUCTION

Oil-induced arthritis (OIA) in DA rats is an experimental model for autoimmune arthritis and is characterized by an inflammatory synovitis with degradation of the articular cartilage and subchondral bone. OIA is a T-cell mediated disease,[1–3] influenced both by major histocompatability complex (MHC) genes[4] and by non-MHC genes.[5] It is induced with a single intradermal injection of mineral oil or mineral oil-derived products[1,6] and can be classified as an adjuvant-induced arthritis model. As with mycobacterial adjuvant arthritis (AA), OIA can be transferred to naive irradiated recipients with mitogen-activated T cells from OIA immunized animals indicating that, regardless of the immunogenic status of the arthritogen, T cells are important in the initiation of arthritic disease.[2]

To explore the possibility that a differential CD4/CD8 T-cell involvement could account for the passively induced OIA, we investigated T cells from OIA immunized rats depleted of $CD4^+$ or $CD8^+$ cells by magnetic cell separation (MACS) for induction of arthritis in naive recipients. In the present report we demonstrate the involvement of $CD4^+$ T cells in the induction of passive OIA.

In vivo administration of monoclonal antibodies (mAbs) to the CD4 molecule, interleukin-2 receptor (IL-2R) or to the intracellular adhesion molecule-1 (ICAM-1) has earlier been used to assess the importance of these cell surface antigens in AA, with the finding that it prevents or suppresses adoptive transfer of AA.[7–9] However, with this treatment protocol not only the transferred cells are targeted but also host-derived immune cells which according to van de Langerijt *et al.*[10] are crucial for arthritis induction in the adoptive transfer system. Therefore, by blocking CD4, IL-2R and ICAM-1 with monoclonal antibodies *in vitro*, we only wanted to investigate the role of these cell surface components on the transferred $CD4^+$

Received 24 October 1996; revised 28 January 1997; accepted 24 February 1997.

Abbreviations: OIA, oil-induced arthritis; IFA, incomplete Freund's adjuvant; mAb, monoclonal antibodies; MACS, magnetic cell separation; ICAM-1, intracellular adhesion molecule-1; TCR, T-cell receptor; LNC, lymph node cells; IL-2, interleukin-2; IL-4, interleukin-4; IFN-γ, interferon-γ; TNF-α, tumour necrosis factor-α; Con A, Concanavalin A.

Correspondence: Dr S. Kleinau, Department of Pathology, University Hospital, S-751 85 Uppsala, Sweden.

arthritogenic T cells in the initiation of adoptive tansfer of OIA.

A characterization of the T-cell receptor (TCR) usage of the $CD4^+$ T cells mediating passive transfer of OIA would allow identification of clones specific for target antigens. By transcriptional analysis of TCR genes we analysed the expression of specific TCR variable (V) β chains by using primers specific to each rat Vβ family.

The $CD4^+$ T cells were also characterized by identifying their expression of T helper (Th)1/Th2 cytokines. The expression of IL-2, interferon-γ (IFN-γ), tumour necrosis factor-α (TNF-α) and interleukin-4 (IL-4) mRNA was investigated by *in situ* hybridization.

MATERIALS AND METHODS

Animals

200 g DA rats of male sex were used. The animals were kept and bred at the animal unit of the Karolinska hospital and were originally obtained from the Central Institute for Laboratory Animal Breeding, Hannover, Germany. The animals were fed rodent chow and water *ad libitum*. The rat colonies were screened for pathogens and were determined to be free from common pathogens.

Donor rats

Donor rats were immunized intradermally with 200 μl incomplete Freund's adjuvant (IFA; Difco, Detroit, MI) at the base of the tail. 13–15 days after injection, popliteal, inguinal, axillary and brachial lymph nodes were removed. For *in situ* hybridization experiments lymph nodes from unimmunized DA rats were also used.

Mitogen-activated T cells

Single cell suspensions were prepared by passing the lymph nodes through a stainless-steel mesh. Lymph node cells (LNC) from several rats were pooled and cultured at $0{\cdot}5–1 \times 10^7$ cells/ml with 3 μg/ml Concanavalin A (Con A) in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2 mM glutamine, 100 μg/ml streptomycin, 100 /ml D-penicillin, 2 M HEPES, 0·1 M β-mercaptoethanol and 10% fetal calf serum. The cells were cultured at 37° in a humidified 6% CO_2 atmosphere for 48 hr. The mitogen-activated LNC were thereafter washed in phosphate-buffered saline (PBS) and put on a nylon wool column to separate the T cells.

CD4 and CD8 cell depletions

$CD4^+$ or $CD8^+$ cells were depleted from the T-cell fraction using a magnetic cell separation system MACS (Miltenyi Biotec, Bergish Gladbach, Germany), as described earlier.[11] Briefly, cells were incubated with biotin-labelled anti-CD4 mAb (W3/25) or biotin-labelled anti-CD8 mAb (Ox8) (Serotec, Oxford, UK) at 50 μg/ml to 5×10^7 T cells for 10 min at 4°. After washing in PBS–1% bovine serum albumin (BSA) the cells were incubated with streptavidin-conjugated paramagnetic microbeads (Miltenyi-Biotec) at 10 μl/10^7 cells for 10 min at 4°. After washing in PBS–1% BSA the cell suspension was passed over an ironwool column within a magnetic field. The magnetic-bead labelled cells (positive fraction) were retained on the column and the unlabelled cells (negative fraction) passed through and were collected. The negative fraction was washed in PBS, counted and transferred to recipients. In each transfer experiment the purity of the resulting CD4 or CD8 depletion was assessed by flow cytometry. The purity of $CD4^+$, TCR $\alpha\beta^+$ cells was between 91 and 98% with 0–0·1% contaminating $CD8^+$ cells and the purity of $CD8^+$, TCR $\alpha\beta^+$ was between 73 and 87% with 0·1–4·9% contaminating $CD4^+$ cells.

Adoptive transfer and evaluation of arthritis

Naive DA rats were irradiated with 600 rad and 24–48 hr later injected intravenously with $0{\cdot}9–7{\cdot}5 \times 10^7$ viable $CD4^+$ or $CD8^+$ T cells in 0·4–2 ml PBS. The recipients were subsequently inspected five times per week for clinical signs of arthritis. Severity of arthritis was graded from 0 to 4 for each extremity as previously described,[1] giving a maximum score of 16.

In vitro *blockage of CD4, ICAM-1 and IL-2 receptor*

Before transfer, $CD4^+$ T cells from IFA-immunized DA rats were either incubated with 3 μg/ml anti-CD4 mAb (W3/25), 20 μg/ml anti-ICAM-1 mAb (IA29) or 10 μg/ml anti-IL-2 receptor mAb (Ox39) (Serotec) for 30 min at 4° and thereafter washed in PBS. Antibody binding was verified by fluorescence-activated cell sorting (FACS) analysis in each experiment.

Polymerase chain reaction (PCR) analysis

Total RNA was prepared from Con A-stimulated T cells obtained from IFA-immunized DA rats. Cells were lysed in guanidinium thiocyanate and extracted with phenol.[12] The isolated RNA was used to synthesize cDNA as template for the PCR reactions, as described elsewhere.[13] Briefly, cDNA was made with a Cβ-specific primer and subsequent PCR reactions were set up with a radioactive-labelled Cβ-primer, internal to the one used in the cDNA synthesis, and one of 22 different Vβ-specific primers. The PCR products were separated in an acrylamide gel and an X-ray film was exposed to the gel. With aid of the X-ray film the bands were excised and the radioactivity measured by liquid scintillation. The signal from each band was expressed as a percentage of the total signal.

In situ *hybridization*

Slides with freshly isolated $CD4^+$ T cells or mitogen-activated $CD4^+$ T cells from IFA-injected DA rats and mitogen-activated $CD4^+$ T cells from unimmunized rats were prepared by cytocentrifugation. 1×10^5 cells were loaded per slide and centrifuged at 80 ***g*** for 3 min. Thereafter the cells were fixed for 1 min in 4% paraformaldehyde in PBS at 4°. Slides were stored in 70% ethanol in diethylpyrocarbonate (DEPC)-treated distilled water at 4° until assayed.

RNA probes for TNF-α, IFN-γ, IL-2 and IL-4, described previously,[14] and labelled by α-^{35}S-uridine triphosphate (UdT) were used. The RNA–RNA *in situ* hybridization was performed as described in Müssener *et al.*[14] Briefly, the slides were washed in 2 × saline sodium citrate (SSC) and acetylated for 10 min with 0·25% acetic anhydride. After rinses in 2 × SSC and PBS, slides were incubated in 1 M Tris–HCl/1 M glycine for 30 min. After additional washes in 2 × SSC, slides were transferred to 50% formamide–2 × SSC at 55°, before the probe was added. Hybridization of cRNA was performed for 16 hr at 52° in a humidified chamber, with one microlitre of ^{35}S-labelled cRNA probe, mixed in 50% formamide, 2%

dextran sulfate, 2 × SSC, 100 mM dichlorodiphenyltrichloroethane (DDT), 1 μg/μl *Escherichia coli* tRNA, 0·5 μg/μl sheared herring sperm DNA, 10 mM vanadyl ribonuclease inhibitor and 2 μg/μl nuclease-free BSA. Slides were thereafter rinsed in 50% formamide–2 × SSC/10 mM DTT, kept at 56°, and then rinsed in 2 × SSC. Single-stranded RNA was digested by 100 μg/ml RNAse A and 1 μg/ml RNAse T1 at 37° for 30 min. After rinsing in 2 × SSC, the slides were rinsed in 50% formamide–2 × SSC at 52°, rinsed again in 2 × SCC, dehydrated in ethanol, and air dried. The slides were covered with NTB-2 nuclear track solution (Eastman-Kodak, Rochester, NY), and developed after 20–25 days exposure. Hybridized cells were counterstained with Giemsa and cells expressing numerous grains were coded as positive. The results were expressed as numbers of labelled cells per 1×10^5 plated cells. Sense probes were used as controls and never gave signal above background.

RESULTS AND DISCUSSION

Transfer of CD4$^+$ and CD8$^+$ T cells

We have previously reported that lymph node cells (LNC) or enriched T cells from lymph nodes of IFA-injected DA rats can transfer OIA to naive recipients.[2] A high disease frequency in recipients was achieved by injecting a minimum of 10^7 Con A-activated LNC or T cells into irradiated animals. The arthritogenic potential of the transferred cells was activated by Con A since non-mitogen (freshly harvested) LNC from IFA-injected DA rats did not transfer arthritis.

In the present study, Con A-activated T cells from IFA-immunized DA rats were depleted of CD4$^+$ or CD8$^+$ T cells, using the MACS system, before transfer into irradiated naive recipients to elucidate the role of T-lymphocyte subsets in the induction of OIA. Passive OIA could be induced in five out of five rats receiving $3{\cdot}5$–$4{\cdot}0 \times 10^7$ mitogen-activated CD4$^+$ T cells from DA rats injected with IFA 14 days previously (Table 1). Arthritis developed within 5–12 days after cell transfer with highest arthritic score ranging from 2 to 7 (Fig. 1). The recipient receiving the highest number of CD4$^+$ T cells developed the most severe arthritis, with the highest arthritic score of 7, which became chronic and lasted for more than 70 days (data not shown).

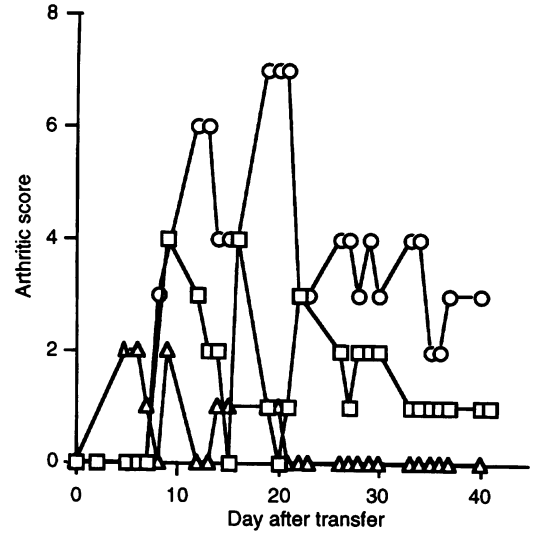

Figure 1. Time course of passively transferred OIA in irradiated recipients. (□) mean arthritic score in recipients of $3{\cdot}5 \times 10^7$ Con A-activated, IFA-sensitized CD4$^+$ T cells ($n=2$). (△) mean arthritic score in recipients of $3{\cdot}6 \times 10^7$ Con A-stimulated, IFA-sensitized CD4$^+$ T cells ($n=2$). (○) arthritic score in recipient of 4×10^7 Con A-stimulated IFA-sensitized CD4+T cells ($n=1$).

Table 1. Incidence of arthritis in irradiated naive DA rats receiving Con A-activated CD4$^+$ or CD8$^+$ T-cells from DA rats immunized with IFA 14 days previously

T-cell subset	Exp. no.	No. of cells transferred $\times 10^7$	CD4$^+$, TCR$\alpha\beta^+$ (%)	CD8$^+$, TCR$\alpha\beta^+$ (%)	No. arthritic/ total no. recipients	Day of arthritis onset	Highest score of arthritis
CD4	1	3·5	91		2/2	9; 12	4; 4
	2	3·6	97		2/2	7; 5	2; 3
	3	4·0	98		1/1	8	7
CD8	4	0·9		84	0/1		
	5	1·3		80*	0/1		
	6	2·5		85	0/1		
	7	4·0		87	1/1	27	7
	8	6·1		73	0/1		
	9	7·5		75	0/1		
Control†					7/7	12‡	8§
Control¶					0/2		

*Only CD8 determined.
†Actively induced OIA. DA rats receiving 200 μl IFA i.d.
‡Mean day of arthritis onset.
§Mean highest score of arthritis.
¶Irradiated DA rats receiving 200 μl IFA i.d.

Transfer of mitogen-activated $CD8^+$ T cells from IFA-injected DA rats in doses of $0{\cdot}9–7{\cdot}5 \times 10^7$ cells induced arthritis in one out of six rats. The development of arthritis in the animal receiving 4×10^7 $CD8^+$ cells was not apparent until 27 days after transfer. In this experiment, however, 4·9% of the total cells transferred were positive for CD4. Since none of the other rats receiving $CD8^+$ cells, although in high numbers, developed arthritis, the high contamination of $CD4^+$ T cells, which could expand in the recipient, might most likely account for the development of arthritis in the one rat receiving $CD8^+$ T cells.

Our observations that the $CD4^+$ subset of T cells are essential for passive OIA is analogous to adoptive transfer of adjuvant arthritis and collagen induced arthritis, in which the $CD4^+$ cells have been discerned as mediators for disease induction.[15–17] Also, prophylactic treatment with monoclonal antibodies to the CD4 molecule on active collagen induced arthritis and adjuvant arthritis have demonstrated that $CD4^+$ cells are involved in the induction phase of experimental arthritic disease.[7,18]

Blockade of CD4, ICAM-1 and IL-2 receptor on $CD4^+$ T cells prior to transfer

We investigated if blockade of the CD4 molecule could inhibit the adoptive transfer of OIA since $CD4^+$ T cells mediated the disease. Interestingly, the arthritogenic $CD4^+$ cells were insensitive to *in vitro* treatment with mAb to the CD4 molecule and induced arthritis in three out of three recipients after transfer with 4×10^7 $CD4^+$ T cells after prior incubation with W3/25 mAb (Table 2). Mean onset of disease was 7 days after cell transfer with a maximal clinical score of 4. Blockage of ICAM-1 had a minor effect on arthritis development, in that three out of four rats developed arthritis with an average onset of 9 days and highest arthritic score of 5 after transfer with 4×10^7 $CD4^+$ T cells from IFA-immunized rats incubated with IA29 before transfer. When similar numbers of arthritogenic $CD4^+$ T cells were treated instead with mAb to the IL-2 receptor before transfer, there was a lower incidence of disease (although not statistically proven) with a delayed onset in the two out of five rats that developed arthritis. Mean day of arthritis onset was 23 days after transfer with a mean maximal clinical score of 3·5. Six control rats receiving 4×10^7 arthritogenic $CD4^+$ T cells without any prior treatment all developed arthritis. The initial signs of arthritis were evident 9 days after transfer, reaching a mean maximal clinical score of 3·8.

The absence of inhibition of passive OIA by blocking the CD4 molecule or ICAM-1 may be explained thus:[1] the monoclonal antibodies dissociate when the cells enter the receipients circulation; or[2] the arthritogenic process is not dependent on the CD4 molecule or ICAM-1. We believe that the former is not very likely because the mAb W3/25 against CD4, or 1A29 recognizing ICAM-1 have earlier been used successfully *in vivo* for adoptive transfer of adjuvant arthritis.[7,9] However, our results may differ due to that in our experiments only the transferred cells and not the recipients were treated with W3/25 or 1A29. Inhibition of passive OIA by treating the cells with mAb to the IL-2 receptor also indicates that the mAb has not dissociated *in vivo*.

It is possible that simultaneous blockade of additional adhesion molecules to ICAM-1 may be neccessary for inhibiting transfer of arthritis, but again 1A29 used *in vivo* alone has earlier been reported to suppress passive AA.[9] Knowing that adhesion molecules are a prerequsite for the leucocytes to attach to endothelium and extravasate into inflammatory foci, our results may indicate that the arthritogenic cells do not themselves migrate to joints and initiate arthritis there. This has also been postulated and studied more directly in adoptive transfer of adjuvant arthritis by van de Langerijt *et al.*[10] who reported that transferred cells localized to liver, spleen and lymph nodes but not to joints.

The inhibition of passive OIA with blockade of the IL-2 receptor suggests that the $CD4^+$ T cells are dependent on IL-2 for survival in the recipient. Our results of the *in vitro* IL-2 receptor-directed treatment parallels the *in vivo* treatment of IL-2 receptor in passive AA which prevents disease.[8]

Cytokine mRNA expression in $CD4^+$ arthritogenic T cells

The next issue was focused on identifying the involvement of Th1 and/or Th2 subsets of T helper cells in the passively induced OIA based on their lymphokine expression patterns. By using *in situ* hybridization, Con A-activated IFA-sensitized $CD4^+$ T cells expressed mRNA for Il-2, IFN-γ and TNF-α but not IL-4 (Table 3) (Fig. 2). IL-2 was markedly expressed followed by IFN-γ and TNF-α mRNA expressing cells. Without prior *in vitro* stimulation of Con A, $CD4^+$ T cells from IFA-immunized rats also exhibited a Th1-like cytokine profile expressing IFN-γ mRNA, although fewer positive cells could be detected compared to control cells stimulated with

Table 2. Transfer of 4×10^7 untreated or mAb treated Con A-activated $CD4^+$ T cells from IFA-injected DA rats to naive irradiated recipients

Treatment (mAb)	No. arthritic/ total no.rats	Mean day of onset	Arthritis severity*
None	7/7	8	3·6
Anti-CD4 (W3/25)	3/3	7	4
Anti-ICAM-1 (IA29)	3/4	10	2·3
Anti-IL-2-receptor (Ox 39)	2/5	23*	3·5

*Data are expressed as mean of the maximal clinical scores of arthritic rats.

*$P < 0{\cdot}05$ versus control group, Student's t-test.

Table 3. Cytokine mRNA expression in $CD4^+$ T cells isolated from lymph nodes of unimmunized or IFA-immunized DA rats 14 days after immunization. The data are depicted as numbers of cytokine expressing cells per 1×10^5 $CD4^+$ T cells

Source of $CD4^+$ T cells	Con A-activation	TNF-α	IFN-γ	IL-2	IL-4
Unimmunized rats	Yes	5	21	116	3
IFA-injected rats	No	0	22	0	0
IFA-injected rats	Yes	300	500	1750	0

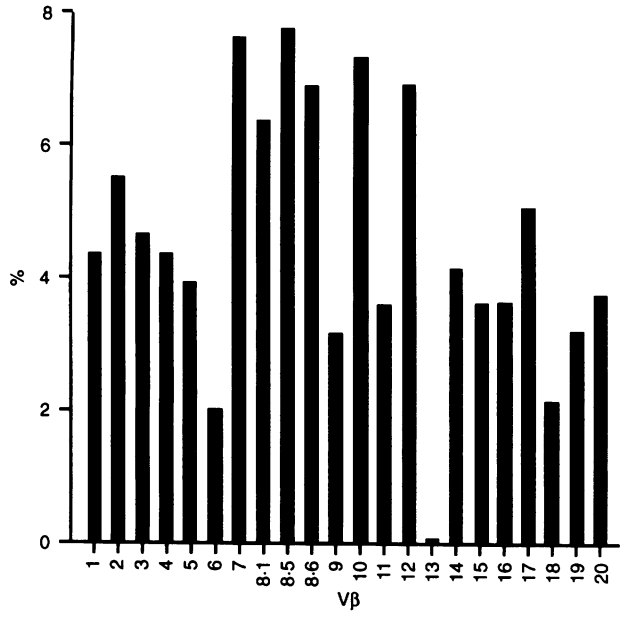

Figure 2. Expression of T-cell receptor Vβ chains in Con A-activated CD4$^+$ T cells from DA rats immunized with IFA 14 days previously and analysed by polymerase chain reaction using 22 Vβ-specific primers.

Con A (Table 3). This indicates that the selective expansion of this subset of T cells had already occurred *in vivo* and was not due to mitogen stimulation.

As a comparison to Con A-activated IFA-sensitized CD4$^+$ T cells, mitogen-activated CD4$^+$ T cells from non-immunized DA rats were analysed, and although very few cells were analysed, IL-4 mRNA was additionally detected (Table 3). The number of cells expressing IL-2, IFN-γ and TNF-α were also about 10-fold lower than in mitogen-activated CD4$^+$ T cells obtained from IFA-immunized rats. These findings demonstrate that non-immunogenic mineral oil in the form of IFA selectively activates a Th1-like subset of T cells in the DA rat.

TCR Vβ repertoire of CD4$^+$ arthritogenic T cells

To assess if Con A may also serve to expand populations of T cells with critical antigenic specificities we investigated the TCR repertoire of the mitogen-activated T cells from OIA immunized rats. The TCR Vβ population was studied by PCR amplification using 22 different Vβ-specific primers corresponding to all known Vβ-genes in rat. As depicted in Fig. 2, amplified products revealed extensive heterogeneity of TcR Vβ, demonstrating a polyclonal Vβ population of the arthritogenic T cells. This means that the arthritogenic cells are not reactive with a single autoantigen or that just a small subpopulation (about 2–8%) of the disease inducing cells recognize a specific target antigen. Concerning T-cell reactivity in OIA to joint candidate autoantigens, earlier studies have failed to demonstrate any reactivity against collagen type II, proteoglycans or 65 000 MW heat shock protein.[1]

The polyclonal picture of arthritogenic T cells in OIA is well in agreement with what has been reported recently in another model of mineral oil-induced arthritis in mice, namely pristane-induced arthritis (PIA).[19] In this study it was observed that the T- and B-cell responses in PIA are to a wide range of targets (multiple joint components).

Altogether, our results indicate that the arthritogenic process in DA rats in response to non-immunogenic adjuvant oil requires the interaction of CD4$^+$ T cells, although the CD4 molecule seems to be of minor importance for the induction of arthritis. An IL-2 dependancy of the arthritogenic T cells was noted, demonstrated by the high expression of IL-2 and the importance of the IL-2 receptor. The observed chronic phase of passive OIA may also be facilitated by the high number of IL-2 expressing cells. In a previous report we have demonstrated that expression of IL-2 in lymph nodes can be observed in DA rats with homologous CIA, which is a chronic disease, but not in lymph nodes from DA rats with non-chronic, actively induced OIA.[14]

The development of arthritis in recipients after transfer with polyclonal T cells expressing Th1 cytokines, without disturbance of blockade of ICAM-1 *in vitro*, indicates that passive OIA is mediated by cytokine-secreting T cells which may activate, recruit or interfere with T cells or macrophages in the receipient, leading to induction of arthritis.

Since the specific antigen that triggers the autoreactive T-cell response in rheumatoid arthritis remains unknown, this study illustrates that autoimmune arthritis need not neccessarily be triggered by T cells recognizing a specific autoantigen.

ACKNOWLEDGMENTS

We thank Dr Robert Harris for rewarding linguistic advice. This work was supported by King Gustav V's 80 year Foundation, Åke Wiberg's foundation, Börje Dahlin's foundation, Nanna Svartz foundation, Alex and Eva Wallströms foundation, Ulla and Gustaf af Ugglas foundation and the Reumatismassociation.

REFERENCES

1. Kleinau S., Erlandsson H., Holmdahl R. & Klareskog L. (1991) Adjuvant oils induce arthritis in the DA rat. I. Characterization of the disease and evidence for an immunological involvement. *J Autoimmunity* **4**, 871.
2. Kleinau S. & Klareskog L. (1993) Oil-induced arthritis in DA rats. Passive transfer of T-cells but not with serum. *J Autoimmunity* **6**, 449.
3. Goldschmidt T.J. & Holmdahl R. (1991) Anti-T cell receptor antibody treatment of rats with established autologous collagen-induced arthritis: suppression of arthritis without reduction of anti-type II collagen autoantibody levels. *Eur J Immunol* **21**, 1327.
4. Cannon G.W., Woods M.L., Clayton F. & Griffiths M.M. (1993) Induction of arthritis in DA rats by incomplete Freund's adjuvant. *J Rheumatol* **20**, 7.
5. Lorentzen J.C., Olsson T. & Klareskog L. (1995) Susceptibility to oil-induced arthritis in the DA rat is determined by MHC and non-MHC genes. *Transplant Proc* **27**, 1532.
6. Kleinau S., Dencker L. & Klareskog L. (1995) Oil-induced arthritis in DA rats: Tissue distribution of arthritogenic ^{14}C-labelled hexadecane. *Int J Immunopharmacol* **17**, 393.
7. Billingham M.E.J., Hicks C. & Carney S. (1990) Monoclonal antibodies and arthritis. *Agents Action* **29**, 77.
8. Stünkel K.G., Theisen P., Mouzaki A., Diamantstein T. & Schlumberger H.D. (1988) Monitoring of interleukin-2 receptor (Il-2R) *in vivo* and studies on an IL-2R-directed immunosuppressive therapy of active and adoptive adjuvant-induced arthritis in rats. *Immunology* **64**, 683.

9. IIGO Y., TAKASHI T., TAMATANI T. *et al.* (1991) ICAM-1-dependent pathway is critically involved in the pathogeneses of adjuvant arthritis in rats. *J Immunol* **147,** 4167.
10. VAN DE LANGERIJT, VOLSEN S.G., HICKS C.A., CRAIG P.J., BILLINGHAM M.E.J. & VAN DEN BERG W.B. (1994) Cell migration studies in the adoptive transfer of adjuvant arthritis in the Lewis rat. *Immunology* **81,** 414.
11. MILTENYI S., MÜLLER W., WEICHEL W. & RADBRUCH A. (1990) High gradient magnetic cell separation with MACS. *Cytometry* **11,** 231.
12. CHOMCZYNZKI P. & SACCHI N. (1987) Single step method of RNA isolation by acid guanidinium thiocyanate–chloroform extraction. *Anal Biochem* **162,** 156.
13. GOLD D.P., VAINIENE M., CELNIK B. *et al.* (1992) Characterization of the immune response to a secondary encephalitogenic epitope of basic protein in Lewis rats. II. Biased TCR Vβ expression predominates in spinal cord infiltrating T cells. *J Immunol* **148,** 1712.
14. MÜSSENER Å., KLARESKOG L., LORENTZEN J.C. & KLEINAU S. (1995) TNF-α dominates cytokine mRNA expression in lymphoid tissue of rats developing collagen-and oil-induced arthritis. *Scand J Immunol* **42,** 128.
15. HOLOSHITZ J., NAPARSTEK Y., BEN-NUN A. & COHEN I.R. (1983) Lines of T lymphocytes induce or vaccinate against autoimmune arthritis. *Science* **219,** 56.
16. KAKIMOTO K., KATSUKI M., HIROFUJI T., IWATA H. & KOGA T. (1988) Isolation of T-cell line capable of protecting mice against collagen-induced arthrits. *J Immunol* **140,** 78.
17. KADOWAKI K.M., MATSUNO H., TSUJI H. & TUNRU I. (1994) CD4$^+$ T cells from collagen-induced arthritic mice are essential to transfer arthritis into severe combined immunodeficient mice. *Clin Exp Immunol* **97,** 212.
18. RANGES G., SRIRAM S. & COOPER S.M. (1985) Prevention of type II collagen-induced arthritis by *in vivo* treatment with anti-L3T4. *J Exp Med* **162,** 1105.
19. BARKER R.N., EASTERFIELD A.J., ALLEN R.F., WELLS A.D., ELSON C.J. & THOMPSON S.J. (1996) B- and T-cell autoantigens in pristane-induced arthritis. *Immunology* **89,** 189.