江苏省首届青年学术年会

论文集

（医科分册）

JIANGSU
1992

江苏省首届青年学术年会执行委员会编

中国科学技术出版社

江苏省首届青年学术年会

论　文　集

（医科分册）

江苏省首届青年学术年会

执行委员会编

JIANGSU
1992

中国科学技术出版社

内 容 提 要

江苏省首届青年学术年会论文集（医学分册）共收录了有较高学术水平的论文73篇、论文摘要30篇，内容涉及基础医学的免疫学、遗传学、生理学、生物化学、病理学、寄生虫学等；临床医学的内科、外科、妇科、儿科、传染病、耳鼻喉科、眼科、护理；预防医学的营养卫生、食品卫生等；药学及中医中药的有关学科。这些论文集中反映了近几年江苏青年医学科技工作者的研究成果，对医学基础、临床、科研和教学工作均有参考价值。

（京）新登字175号

江苏省首届青年学术年会论文集

（医科分册）

江苏省首届青年学术年会

执行委员会 编

*

中国科学技术出版社出版发行（北京海淀区白石桥路32号）

江苏省新华印刷厂印刷

*

开本：787×1092毫米 1/16 印张：119 字数：2800千字

1992年12月第一版 1992年12月第一次印刷

印数：1—1000册 全套定价：134.00元

ISBN 7-5046-0932-3/Z·88

江苏省首届青年学术年会
指导委员会名单

主　　席：吴锡军
副 主 席：孙钟秀　潘　恕
秘 书 长：王世璜
副秘书长：李茂典
委　　员：李庆逵　严　恺　钱钟韩　冯　端　窦国仁
童　傅　韦　钰　高亮之　余之祥　陈家伟
郭克礼　葛锁网　王永顺　潘宗白　陈　懿
何立权　王臻中　叶钟音　陈荣华

江苏省首届青年学术年会
执行委员会名单

主　　席：尤肖虎
副 主 席：张　荣
秘 书 长：张铁恒
副秘书长：王安宁
委　　员：王兆球　王建国　沈其荣　王心如　李剑波
梁德旺　毛罕平　姚　远　刘　平　钱海鑫
顾晓松　郭　荣　葛世荣　裘　伟　王明秋
杨善德　朱永官

前　言

当今时代，科学技术已成为决定生产力的首要因素，社会经济的发展更多地依赖于科学技术的进步，依赖于掌握先进科学技术的人。青年科技工作者作为跨世纪的科技生力军，必将并且正在成为我国科技队伍的骨干力量。有计划地培养和造就一支强大的、高水平的青年科技中坚力量，是一项重要的战略任务。江苏省科学技术协会主办的江苏省首届青年学术年会，旨在检阅江苏省青年科技工作者的科技成果，发现和培养优秀青年科技人才，推动全社会进一步形成关心、重视青年科技人才成长的良好环境，激励广大青年科技工作者奋发图强、建功立业。

本届年会的征文工作得到全省青年科技工作者以及海外留学生的大力支持，共收到应征论文2000余篇，其中来自美国、日本、英国、法国、加拿大、德国、菲律宾等国家的论文20余篇，内容涉及了理、工、农、医、交叉等学科领域，作者大部分为具有高、中级职称或博士、硕士学位的青年科技工作者，反映了江苏省乃至全国青年科技工作者的科技水平。应征论文经年会所聘请的专家委员会反复筛选，从中精选出320余篇，分理、工、农、医、交叉学科五部分汇编成论文集出版发行。由于论文集篇幅所限，许多优秀论文只好割爱。

本届年会得到了中共江苏省委、江苏省人民政府及有关部门的高度重视，得到了江苏省省级学会、各市人民政府、各市科学技术协会及所属学会、各高等院校、研究院所及有关单位的大力支持，特别是得益于南京大学、东南大学、南京农业大学、南京医学院、南京师范大学的积极协助和具体承办。论文集出版过程中承蒙中国科学技术出版社、江苏省新华印刷厂全力支持，并且有一大批热心的同志付出了辛勤劳动，籍此机会一并表示诚挚的谢意。

本书为江苏省首届青年学术年会论文集的医学分册，是在江苏省、市医学会、高等医药院校、解放军医疗卫生单位等初审推荐的518篇论文基础上，由专家评审委员会选出的73篇优秀论文、30篇摘要汇编

而成。内容围绕国家卫生工作重点及江苏省医学科研攻关项目，追综世界医学科技发展新动向，侧重与国民经济密切相关的医药保健及有关基础研究领域，注重医学的综合性和专题性、宏观和微观、理论和实践，体现了江苏省青年医学科技工作者的科学精神和学术水平。参加医学专家评审委员会的成员有王敬良、周志耀、黎介寿、杜竟辉、熊宗璠、杨玉、王廷芳、陈解民、单祥年、姚堃、陈德华、贾辅忠、黄志勇、王毓三、谈瑗声等先生，我们在此表示衷心感谢。

本分册的编辑工作由江苏省首届青年学术年会执行委员会医科分部的王心如、王虹、朱昌亮、沈建、黄煌、魏鹏等同志集体完成。印刷出版工作得到胡晓河、刘福在、禹正瑜等同志协助。由于时间仓促及编辑水平有限，难免会出现错误，希望读者予以谅解。

江苏省首届青年学术年会执行委员会医科分部

1992年10月22日

目　录

摘　要　部　分

抗 IL-6 单抗治疗中出现大量 IL－6 免疫复合物的积聚——一种细胞因子体内生成定量法

陆肇阳① Bataille R. Klein B.
(Plateau technique du CHR, BP1005, quai Moucousu, 44035 Nantes, France)
Brochier J.
(INSERM U291, 99 Rue Puech Villa, 34100 Montpellier, France)
Wijdenes J.
(Centre Regional de Transfusion sanguine, 25020 Besancon, France)
Brailly I.
(Immunotech, Luminy Case 915, 13288 Marseilles Cedex 9, France)

摘要 我们曾报导用抗 IL－6 单抗治疗浆细胞白血病，在治疗末期，病人体内出现大量能刺激 B9 杂交瘤增殖的活性(B9－SA)。本文采用凝胶过滤及亲和层析等方法研究了该 B9－SA。结果表明，进入体内的抗 IL－6 单抗阻断了 IL－6 与其靶细胞膜受体的结合，同时由于 IL－6/抗 IL－6 单价免疫复合物的形成妨碍了肾脏对 IL－6 的排泄，最终导致大量 IL－6 复合物在循环中积聚。由于肿瘤细胞中对细胞因子敏感的亚克隆被选择性地扩增，从而给病人带来新的潜在危险。因此，我们认为单用一种抗细胞因子单抗治疗某些疾病时应慎重。我们还首次推导了一个测定体内 IL－6 日生成量的数学模型(该病人 IL－6 的日生成量约为 15μg)。本结果对细胞因子的临床应用具有重要意义。

1 Introduction

We, and others, have shown that IL-6 is a major myeloma-cell growth factor in patients with active disease, especially in patients with terminal disease[1,2]. These observations led us to make a clinical trial with anti-IL-6 monoclonal antibodies (mAb) in a patient with terminal disease; it resulted in a complete blockage of myeloma-cell proliferation for one-and half month in vivo[2]. In addition, the production of C reactive protein (CRP), an acute-phase protein whose production by human hepatocytes is totally controlled by IL-6 in vitro[3], was competely inhibited. At the end of the anti-IL-6 treatment, high levels of an activity stimulating the proliferation of the B9 hybridoma was detected[2]. The present study was designed to explore the mechanisms responsible for this phenomenon.

2 Materials and methods

① 苏州医学院

One patient with secondary plasma-cell leukemia was treated with anti-IL-6 mAb. The patient was treated for two months, first with the anti-IL-6 B-E4 mAb for 6 days and subsequently with the anti-IL-6 B-E8 mAb until day 67 following the beginning of treatment. On day 82, the patient received a new chemotherapy regimen.

The anti-IL-6 mAb (B-E4, IgG2b and B-E8, IgG1) were prepared by J. Wijdenes by immunizing mice with recombinant IL-6[3]. The two mAb recognize different epitopes on the IL-6 molecule and the B-E8 mAb was about five times more potent than the B-E4 mAb in inhibiting the biological activity of IL-6[4].

5,000 B9 cells[5] were cultured with serial dilutions of the sample in 96-well, flat-bottomed culture dishes in 100 ul of RPMI 1640 culture medium containing 5% FCS. After an incubation period of three days at 37 in 5% CO_2, the cells were exposed to MTT (Sigma, St Louis, MO) for 4 hours at 37[6]. One Unit of B9-stimulating activity (B9-SA) is defined as the amount inducing half-maximal optical densities and corresponds to approximately 5 pg of recombinant IL-6.

Affinity chromatography

For the protein A-Sepharose column, the samples were run with 50 m*M* PBS, pH 8. 1. The bound material was eluted with 0. 1 *M* glycine-HCl, pH 2. 4. For the other columns, the washing and eluting buffers were 50 m*M* PBS, pH 7. 2, and 0. 1 *M* glycine-HCl, pH 2. 3, respectively. Each fraction was immediately neutralized with 1 *M* $NaHCO_3$ and was then assayed for B9-SA at different dilutions.

A 4 ml sample was loaded onto a Sephadex G-200 column (100 cm x 2. 6 cm) and was chromatographed at 4℃ with 50 m*M* PBS (pH 7. 2) as eluting buffer. To dissociate expected immune complexes, the samples were dialyzed for 12 hours with a 0. 1*M* glycine-HCl buffer (pH 2. 3) and were subsequently gel filtrated with the same acid buffer. Each fraction was neutralized with 1*M* $NaHCO_3$ and assayed for B9-SA at different concentrations.

Mathematical procedure for determining the whole daily production of IL-6

The in vivo situation is modeled by a system comprising two compartments, as illustrated in Figure 1. For the anti-IL-6 mAb compartment, let y^+ = the influx of anti-IL - 6 mAb resulting from the daily injection of mAb; y^1 = the efflux of free mAb; y^2 = the efflux of IL-6-bound mAb; mAb_1 = the serum concentration of free mAb; mAb_2 = the serum concentration of IL-6 - bound mAb; mAb/day = the amount of daily injected mAb; V = plasma volume. Under steady state conditions (hypothesis 1); $y^+ = y_1^- + y_2^-$ (equation 1), mAb1 and mAb2 are constant, and $mAb/day = V\int_0^{1day} y^+ \, dt$.

In addition, the influx and efflux of mAb can be expressed as; $y1^- = k_1 mAb_1$ and $y2^- = k_2 mAb_2$ where k_1 and k_2 are constant. In the case of monomeric immune complexes (hypothesis 2), the clearance of these complexes is similar to that of the free mAb[7], and then $k_1 = k_2$. This hypothesis is verified for the B-E8 mAb ([4] and the present study). $y2^- = y^+ + mAb_2/(mAb_2 + mAb_1)$ (equation 2). Concerning the IL-6 compartment, let x^+ = the influx of circulating IL-6 due to the production of IL-6, x^- = the efflux of circulation IL-6 in the form of immune complexes, [IL-6] = serum concentration

of IL-6, and IL-6/day=daily production of IL-6. Thus IL-6/day= $V\int_0^{1day} x^+ dt$. In the present study, the anti-IL-6 mAb was injected in sufficient amounts to completely block the binding of IL-6 by the cell surface receptor as well as its renal elimination . By assuming that there is no other route of elimination of IL-6 than in the form of immune complexes (hypothesis 3) and in case of steady state conditions (hypothesis 1): $x^+ = x^- = y_2^-$. Thus, IL-6/day $= V\int_0^{1day} x^+ dt = V\int_0^{1day} x^- dt = V\int_0^{1day} y_2^- dt$. This equation combined with equation 2 gives: IL-6/day $= V\int_0^{1day} y^+ dt\ mAb_2/(mAb_2+mAb_1)$, or IL-6/day $=$ (mAb/day) $mAb_2/(mAb_1+mAb_2)$. In the present study, mAb_1+mAb_2 = total concentration of mAb. In addition, as all the circulating IL-6 is bound to the anti-IL-6 mAb (as demonstrated in the following), one have $[IL\text{-}6]=mAb_2$. Thus, IL-6/day= (mAb/day) [IL-6]/[mAb]

3 Results

3.1 Serum levels of B9-stimulating activity (B9-SA) in the patient treated with anti IL-6 mAb

No serum B9-SA was found prior to treatment. After injections of the anti-IL-6 B-E4 mAb, high serum B9-SA levels were found and lasted for 6 days (Figure 1). This activity was completely neutralized by the two different anti-IL-6 mAb which recognize different epitopes on the IL-6 molecule (Figure 2). No serum B9-SA could be detected throughout the treatment with the B-E8 mAb[4]. When the injection of B-E8 was stopped, very high serum B9-SA was detected again, especially on day 80, when no more circulating anti IL-6 mAb could be detected ([2] and Figure 2).

3.2 Evidence that the B9-SA present in day-80 and day-4 sera is IL-6 bound to the anti-IL-6 mAb

All the B9-SA present in the day-80 serum of the patient was retained on a protein A-sepharose column. After gel filtration on a Sephadex G-200 column, all the B9-SA present in the day-80 serum eluted in a peak of 185,000 MW whereas natural IL-6, eluted in a 25,000 MW peak . When the day-80 serum was treated with a pH2. 3 buffer and was chromatographed at the same pH, all the B9-SA eluted in a 25,000 MW peak similar to natural IL-6 . These experiments indicated that the B9-SA present in the day-80 serum was IL-6 bound to an immunoglobulin molecule. Since no more anti-IL-6 mAb could be detected in this serum, we investigated whether the immunoglobulin molecule bound to IL-6 was a human or mouse immunoglobulin. It was found that all the B9-SA contained in the day-80 serum was retained on anti-mouse IgG-sepharose. This clearly indicated that the IL-6 molecules contained in the day-80 serum were bound to residual murine anti-IL-6 B-E8 mAb, which were in too low a concentration to be detected by ELISA (Figure 1). Similar results (not shown) were obtained for the B9-SA present in the day-4 serum.

3.3 IL-6 accumulated in the form of immune complexes throughout the anti-IL-6 treatment

Whereas no B9-SA was found in the day-26, day-40, day-60 sera of the patient (Figure 1), significant B9-SA could be detected after acid treatment and gel filtration of these sera. Furthermore, this B9-SA also eluted in a 25,000 MW peak. No IL-6 activity could be detected after gel filtration at pH 2. 3 of the patient' s serum before anti-IL-6 treatment (results not shown). The titration of the IL-

6 activity in the different fractions obtained after gel filtration at acid pH made it possible to estimate the total IL-6 activity bound to the circulating mAb in these different sera (Table 1). An approximate 50% loss of IL-6 activity due to acid treatment was also found in the day-80 patient serum.

Table 1 Estimation of the daily production of IL-6 in the patient treated with anti-IL-6 mAb

Days after beginning the treatment	4	40	60	80
Concentration of anti-IL-6 mAb(μg/ml)	45[1]	7.5[2]	10[2]	ND[3]
Serum IL-6 activity bound to anti-IL-6 mAb (U/ml)[4]	624	960	1150	1020
Corrected serum IL-6 activity (U/ml)[5]	1248	1920	2300	2040
Serum concentration of IL-6(ng/ml)[5,6]	7.43	11.43	13.63	12.14
Ratio of IL-6 bound mAb to free mAb(x1000)[7]	0.55	4.9	4.4	NE[8]
Daily injection of anti-IL-6 mAb (mg)	40[1]	10	10	0
Daily production of IL-6 (μg)	NE[8]	15.2	13.6	NE[8]

1 B-E4 mAb; 2 B-E8mAb; 3 ND=not detectable;

4 The serum IL-6 activity was determined following gel filtration at pH 2.3;

5 The serum IL-6 activity was multiplied by 2, since the loss of IL-6 activity due to gel filtration was estimated to be about 50%;

6 we determined that 1 U of IL-6 activity in the B9 hybridoma assay corresponded to 5 pg of rh-IL-6 (MW 21,000). It thus corresponded to 5 x (25,000/21,000) pg of natural IL-6 since the natural serum IL-6 was found to have a 25,000 MW.

7 The MW of the two anti-IL-6 mAbs was 160,000 and we considered that one anti-IL-6 mAb molecule has two potential IL-6 binding sites.

8 NE:non-valuable.

3.4 Estimation of the total daily production of IL-6

Indeed treatments at pH 2.3 and gel filtration allowed measurements of IL-6 activity, bound to the anti IL-6 mAb, to be made and, in consequence, allowed us to determine the ratio of IL-6-bound mAb to free mAb in these sera (see detailed explanations in Table 1). This made it possible to establish a minimal estimate of the daily production of IL-6 in the patients, according to the mathematical procedure detailed in the "Materials and Methods" section. One of the necessary three assumptions was that the system was in steady state. This assumption was verified for the day-40 and day-60 sera since a constant amount of anti-IL-6 mAb was injected daily and a stable serum mAb concentration was reached. It was not verfied for the day-4 and day-80 sera ([12] and Table 1). In consequence, the estimate was made only for the day-40 and day-60 sera; it ranged around 15 μg of IL-6 per day (Table 1).

4. Discussion

Recombinant IL-6 has also a half-life of few minutes in the serum and ,similar to IL-2[8],is mainly bound by cell surface receptors (primarily in the liver)and is also eliminated by renal filtration[9].

IL-6 is overproduced in patients with active MM. In the patient with terminal MM who was treated by anti-IL-6 mAb,the efficacy of the mAb in preventing IL-6 binding to its receptor in vivo was demonstrated by the complete inhibition of CRP production and by the blockage of myeloma-cell proliferation[2]. This study shows that IL-6 accumulated in the plasma of this patient in the form of monomeric immune complexes(i. e. 185 kDa MW) as a consequence of this non-absorption by IL-6 receptors. Moreover, the formation of these monomeric immune complexes stopped IL-6 from being eliminated by renal filtration.

This finding is of general interest for the in-vivo use of cytokinebinding molecules,especially in cancer patients. The technique may represent a potential danger by mobilizing high levels of cytokine, in the form of complexes,close to tumoral subclones hypersensitive to the cytokine, and which,unlike normal cells,may be able to dissociate these complexes. This is all the more evident in the case of monomeric immune complexes which should have a long half-life in vivo similar to that of the free B-E8 mAb (3-4 days)[2],predicted by data of the literature[7],and as directly demonstrated by one of us by studying pharmacokinetic of radiolabeled IL-6 in animals pretreated with the B-E4 or B-E8 mAb (H. Brailly,unpublished observations). This may explain why the patient became resistant to the 2-month anti-IL-6 treatment whereas serum CRP levels remained undetectable and in-vitro tumor proliferation remained dependent upon IL-6[2]. The present findings did not encourage us to pursue such anti-IL-6 therapy in patients with MM using a single antibody. Alternative strategies that did not lead to accumulation of monomeric immune complexes should be developed.

The second important point is the phenomenon was due to the decrease of the anti-IL-6 mAb serum concentration to undetectable levels and ,as IL-6 was continuously produced in vivo, to the increase of the ratio of IL-6-bound mAb to free mAb. This allowed the dissociation of these immune complexes by the IL-6 receptors of the very sensitive B9 hybridoma. This again emphasizes the need to maintain a high anti-IL-6 mAb serum concentration during anti IL-treatment.

Finally, we show that this observation leads to a simple methodology for estimating the total in-vivo production of cytokines. The extent of this production has never heen evaluated before. In particular, the serum level of these cytokines, when detectable, reflects only a minor part of their overall synthesis. The mathematical procedure which we developed is based on three assumptions,two of them being verified. First, the system is in steady state. As indicated above,this assumption was verified for days 40 and 60 of treatment. Second, the half-life of the IL-6/anti-IL-6 mAb complexes should be similar to that of anti – IL-6mAb. This assumption is verified for monomeric immune complexes[7]. and that was the case of the IL-6/B-E8 mAb complexes which had a 185 kDa MW. Third,there should be no route of elimination of IL-6 other than in the form of immune complexes. These assumptions were obligatory for the methodology and have possibly led us to an underestimation of the daily production of IL-6, estimated to range around 15 μg per day. It is striking that this estimate is not too far from the minimal daily dose of recombinant IL-6 known to be active in animals (1-5 μg/kg)[10-12].

Figure 1

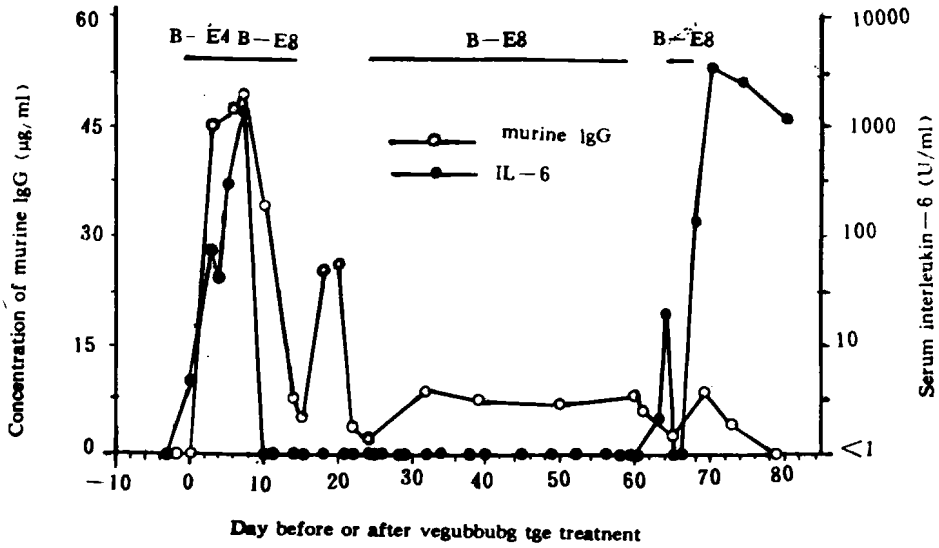
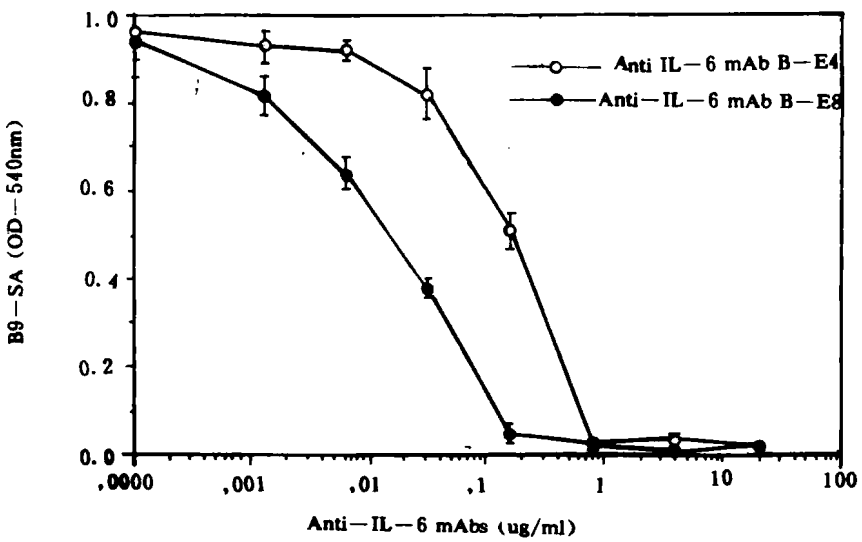


Figure 2



References

1 Klein B, et al. ,Blood 1989;73:517

2 Klein B,et al. ,R. Blood 1991. 78:1198

3 Castell JV,et al. ,Hepatology 1990;12:1179

4 Wijdenes J,et al. ,Molecular Immunology 1991;28:1183

5 Aarden LA,et al. , Eur J Immunol 1987;17:1411

6 Mosmann T. J. ,Immunol Methods 1983;65:55

7 Plotz PH,et al. ,Molecular Immunology 1979;16:721

8 Konard MW,et al. ,Cancer Res1990;50:2009

9 Castell JV. et al. ,Eur. J. Biochem1990;189:113

10 Mayer P,et al. , Exp. Hematol1991;19:7

11 Asano s, et al. ,Blood 1990;75:1602

12 Takatsuki F, et al. ,Cancer Res 1990;50:2885

作者简介 陆肇阳，男，1958 年生。1982 年毕业于苏州医学院，1987 年获硕士学位，同年分配在本院病理生理教研室，1990 年赴法国攻读博士学位。从事肿瘤免疫、细胞因子在肿瘤发病机制中的作用及其临床应用的研究。