·实验研究·

中华猕猴桃多糖的免疫药理学作用

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内容提要　中华猕猴桃根是一种中药材。本文报道了从它的根中提取的多糖复合物(简称ACPS)对小鼠免疫系统的调节作用。小鼠每日腹腔注射 ACPS，1～2 周后分别测定免疫功能。结果：(1)ACPS 能明显促进天然杀伤(NK)细胞对 YAC－1 淋巴瘤细胞的细胞毒作用；(2)能加强巨噬细胞的吞噬功能；(3)能明显增加特异花结形成细胞(SRFC)数。但对抗体形成细胞 (PFC) 无任何影响；(4) 能有效地恢复被环磷酰胺抑制了的迟发超敏(DTH)反应。上述结果表明ACPS是一种有效的免疫调节剂。这在机体抗病毒抗肿瘤中或许有重要意义。

我们曾证明中华猕猴桃多糖复合物 (Actinidia Chinensis Planch Polysaccharide,简称ACPS)能抑制小鼠移植瘤的生长，延长荷瘤鼠的生存期，并使小鼠获得对瘤细胞再次攻击的抵抗力(待发表资料)。本文就 ACPS 的免疫药理学作用及其免疫抗癌机理进行探讨。所获得的结果报道如下。

材料和方法

一、动物：供特异花结形成细胞及溶血空斑形成细胞试验用的均为 DBA/2 纯系小鼠。供测定迟发型超敏反应用的小鼠为 DBA/2 及 NIH 品系，体重18～23g。供测定天然杀伤细胞活性及巨噬细胞吞噬功能的小鼠为BALB/C品系，9～10周龄。

二、药物：ACPS，5 mg/ml，由本所提取。从中华猕猴桃药用部位中获得，经微孔滤膜过滤，冷藏备用。其化学组成70%以上为多糖，并含少量蛋白质。

三、免疫学试验：

1. 抗体形成细胞试验：小鼠每日以 80mg/kg的剂量，腹腔注射 ACPS。于试验前 4 天，即给药后第 5 天，腹腔注射 5 %的羊红细胞 0.4ml。试验时取小鼠脾脏制成匀浆，过 100 目尼龙网，校细胞浓度至 1×10^7 细胞/ml。用细胞介导的红细胞溶血分光光度法，测定空斑形成细胞(PFC)。简言之，取上述脾细胞悬液 1 ml，加入等量的 0.2%羊红细胞及 1∶10 稀释的豚鼠补体。于37°C 保温 1 小时。2000rpm 离心 10 分钟。取上清液于波长 413nm处测光密度。

2. 特异花结形成细胞 (SRFC)的测定[1]：取上述制备的小鼠脾细胞悬液，制成 8×10^6细胞/ml。取 0.2ml 加入等量的1%羊红细胞及小牛血清(先经羊红细胞吸收)。1000rpm离心 10 分钟。于 4 °C 放置 2 小时。每管加 1 %甲苯胺蓝 20μl，轻打混匀，滴于白细胞计数板上，在高倍镜下计数 1 mm^3的 SRFC 数。每个有核细胞结合 5 个以上羊红细胞者为 SRFC。最后换算成 SRFC数目/10^6脾细胞。

3. 天然杀伤(NK) 细胞活性测定[2]：取两种剂量(31.0mg及3.1mg/kg)给小鼠每日腹腔注射。于给药第 10 及 13 天分别取脾脏，按上述方法，制备脾细胞悬液。使细胞浓度为 $1\sim2\times10^7$/ml。取YAC-1 小鼠淋巴瘤细胞作为靶细胞。预先用^{125}IudR(5'-碘脱氧尿嘧啶核苷)标记 2 小时(5～10×10^5细胞/ml 加 10μci ^{125}IudR10μl)。经离心洗涤后配成 1×10^5/ml 细胞浓度。使每个细胞的放射活性约为 1～2 CPM。按效应细胞与靶细胞之比为 200∶1 与 400∶1，将脾细胞与 YAC-1 靶细胞分别混合。终反应体积为 1 ml。每一样品均重复 3 份。经低速离心 2 分钟后，置 CO_2 培养箱37°C培养16～18小时，于 r 计数器上分别测定上清液和细胞沉淀物的放射性脉冲数。在本实验条件下，YAC-1 瘤细胞的自发释放率通常在 11.6±0.6%。实验结果以^{125}IudR释放百分率表示。按下述公式计算：

$$\text{IudR释放}\% = \frac{\text{上清液总CPM}}{\text{细胞CPM}+\text{上清液总CPM}} \times 100$$

4. 巨噬细胞吞噬功能试验：小鼠以 31.0mg/kg 每日腹腔给药共12天。末次给药后24小时，每只小鼠腹腔注射 0.5ml 红酵母菌(1 $\times10^9$菌/ml)。30分钟后，腹腔注射 RPMI-1640 培养液 (pH=7.0) 2 ml。收集腹腔渗出细胞，滴于盖玻片上，移至CO_2培养箱，37°C培养30分钟。染色后显微镜下检查 200 只巨噬细胞，计数吞噬酵母菌的巨噬细胞数及吞噬酵母菌的数目。

求出吞噬细胞百分率及吞噬指数。

5.迟发型超敏(DTH)反应的测定：按 Titus 等方法稍改良[8]。小鼠每日腹腔给药，ACPS按80mg/kg，环磷酰胺(CYT)按100mg/kg，连续5日。于测定前9日，每只小鼠尾根部皮下分二点注射30μl内含0.25%牛血清白蛋白的 Freund 完全佐剂，作为致敏。于致敏后8日，用热凝聚的牛血清白蛋白作足垫皮下攻击，每只小鼠 30μl。24小时后用千分卡尺（精密度0.02mm）测定小鼠右左足垫的厚度。每只测二次，取均值。二足厚度之差，即为肿胀程度。此外，我们在先前的小鼠抑瘤试验中，取同批鼠测定了脾系数（脾重mg/10g体重）。

结　果

一、ACPS 对 SRFC及 PFC 反应的影响：实验分两批，每批动物20～22只，同时测定 SRFC与PFC。两批结果证明：（1）在用羊红细胞初次免疫后所出现的免疫特异花结细胞(SRFC)，对照组为10200±2300 /10^6脾细胞，给药组为 27800±5900/10^6 脾细胞。其值给药组为对照组的272%，P＜0.001，差别甚为显著。(2)与此同时，两组的 PFC 数量相近。对照组(M±SD，以下及各表同）为 8.3±0.96/10^6 脾细胞，给药组为 7.8±0.85/10^6 脾细胞，P＞0.05，两组无差异，见表1。表明 ACPS 能刺激免疫反应早期的抗原结合细胞的增生，但不影响抗体形成细胞，因而 ACPS 在早期所刺激的 SRFC，可能是 T 细胞而不是B细胞。

表 1　体内给予ACPS对SRFC及PFC反应的影响

组　别	SRFC/10^6脾细胞	P	PFC/10^6脾细胞	P
对　照	10200±2300 (22)		8.3±0.96(21)	
ACPS	27800±5900 (22)	＜0.01	7.8±0.85(21)	＞0.05

（　）内表示动物数量

二、ACPS 对 DTH 反应及脾系数的影响：表2的结果显示：CYT组小鼠对致敏牛血清白蛋白的足垫肿胀反应明显受到抑制，但当 CYT 加用 ACPS 后情况就不同，足垫肿胀反应明显增加。增加幅度为单独CYT 组的535～2910%，并达到乃至超过正常对照组。表明 ACPS 能解除由 CYT 造成的免疫抑制状态。单独 ACPS 组的足垫肿胀反应虽超过正常对照组，但两者无显著性差异。

此外，体内给予 ACPS 后亦影响脾系数。对照组小鼠11只平均脾系数为 63.8±17.4；而给药组小鼠 19只平均脾系数为102.1±20.6。两组差异很明显。

三、ACPS体内给药对 NK 细胞活性的影响：于给药的第10、13天，每个剂量组及对照组各取2只BALB/C小鼠，分别测定它们的脾 NK 细胞活性，结果如表3所示。于给药第10天，当效应细胞与靶瘤细胞之比为400：1时，给药组小鼠的 NK 细胞对瘤细胞的杀伤作用明显加强。ACPS 两个剂量组的杀伤率分别为23.9±0.2 与 26.5±2.6，而对照组为 16.4±2.0。给药与对照相比，有非常显著差异。第13天的情况也一样。当效靶细胞之比为200：1时，给药两个剂量组的杀伤百分比亦明显加强。给药与对照相比亦有明显差异。表明 ACPS 体内能激活 NK 细胞的活性。

表 2　体内给予ACPS对迟发超敏反应的影响

组　别	动物数量		足垫肿胀（0.1mm）		P 值	
	NIH	DBA/2	NIH	DBA/2	NIH	DBA/2
CYT	7	4	0.10±0.18	1.07±1.32	标　准	标　准
ACPS	10	6	3.53±1.45	5.52±3.31	＜0.01	＜0.05
CYT +ACPS	10	3	2.91±0.48	5.73±0.97	＜0.01	＜0.01
正常对照	9	8	2.54±1.04	4.97±0.60	＜0.01	＜0.01

表 3　体内给予ACPS对脾脏NK 细胞活性的影响

给药天数	剂　量 (mg/kg)	特异细胞毒作用的百分率 效靶细胞之比 200:1	P 值	400:1	P 值
10	0	17.2±0.5	—	16.4±2.0	—
	3.1	19.8±1.4	＜0.05	23.9±0.2	＜0.01
	31.0	21.9±1.0	＜0.01	26.5±2.6	＜0.01
13	0	31.8±1.1	—	41.2±1.2	—
	3.1	39.9±3.1	＜0.01	50.3±1.8	＜0.01
	31.0	39.2±0.5	＜0.05	47.9±1.6	＜0.05

四、ACPS体内给药对巨噬细胞吞噬功能的影响：小鼠给药12天后，吞噬酵母菌的腹腔巨噬细胞百分率为37.5±9.0，对照组为15.0±4.8%，两组差异显著，P＜0.01。结果见表4。

表 4　体内给予ACPS对巨噬细胞吞噬功能的影响

	吞噬细胞百分率	P	吞噬指数	P
对照	37.5±9.0(8)		0.28±0.13	
给药	15.0±4.8(10)	＜0.01	0.92±0.67	＜0.05

（　）内为动物数量

此外，在显微镜检查时发现每个视野下的巨噬细胞密度，给药组明显高于对照组。而且给药组巨噬细胞所吞噬的酵母菌有些已被消化，仅残留菌体残迹。以上均表明 ACPS 在体内能激活巨噬细胞，增强其吞噬功能。

讨　论

到目前为止，尚未报道中华猕猴桃多糖的免疫调节作用。本文所获得的结果证明：（1）它能促进初次免疫小鼠脾脏中抗原结合细胞的增生。小鼠的这种免疫特异花结细胞，即SRFC，一般包括 T 细胞与 B 细胞。但 ACPS 并不增加抗体形成细胞。因此 ACPS 可能在免疫反应的早期主要调节 T-RFC 的形成。（2）ACPS能诱导和加强 NK 细胞的天然杀伤活性。鉴于NK细胞在机体天然抗肿瘤抗病毒感染方面担负主要角色[4,5]，使寻找 NK 细胞诱导药物，为许多学者所重视[6]。因此，ACPS 的这一作用具有重要意义。

3）ACPS 能解除化学抑制剂对 DTH 反应的抑制作用。一般认为，参与 DTH 反应的细胞为 T_{DTH} 淋巴细胞。据此判断 ACPS 可能对 T_{DTH} 细胞有某种保护作用，或在某个环节上阻断了 CYT 对T_{DTH}的抑制作用。这一点对临床肿瘤化疗也有一定实际意义。因为大多数肿瘤患者的 DTH 反应降低，化疗后更甚，从而使机体对癌细胞失控。（4）ACPS能激活巨噬细胞，促进它的吞噬功能。业已证明激活的巨噬细胞，主要为I_a^-亚群，具有抗瘤活性[7]。以前我们已观察到ACPS对小鼠肿瘤有抑制作用，因而推测 ACPS 所激活的巨噬细胞可能是具有杀瘤作用的某一个亚群。由于ACPS能激活巨噬细胞和 NK 细胞，因而初步揭示了它的免疫抗瘤机理。

最近我们又证实中华猕猴桃的清热解毒作用，即它所含有的 ACPS 具有由免疫介导的抗感染作用。因此，从中医扶正祛邪治则出发，评价中华猕猴桃多糖的作用时，我们愿意指出：ACPS 的扶正作用主要表现为增强机体的免疫功能，特别是细胞免疫功能；它的祛邪作用表现为抗肿瘤、抗细菌感染作用。所以我们认为中华猕猴桃多糖是一种新型的扶正祛邪剂，它不失为一味新的补益药。同时它是一种新的免疫调节剂，尤其是对 NK 细胞的调节，值得从激活细胞的分子产物中加以深入研究。

（中国科学院上海细胞生物所王球达同志，协助测定NK活性，谨此致谢）

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“脾虚证胃病研究”取得可喜进展

无锡市第三人民医院尹光耀医师等，近年来潜心脾虚证胃病的研究取得可喜进展，受到国内许多专家的好评，不久前在北京通过成果鉴定。

该项研究从分子生物学角度探讨脾虚证实质，有助于对脾虚证的中西医结合理论的研究。研究结果表明：（1）环核苷酸、细胞免疫可作为“脾”本质研究的客观指标；（2）不完全性结肠型肠化生可能是引起“肠型胃癌”的重要病理基础；（3）测定环核苷酸可有助于脾虚证分型和肠化生程度的预测；（4）临床发现脾虚气滞证和血浆 cAMP 降低，伴胃粘膜不完全性结肠型肠化，应高度警惕癌变倾向。

专家们认为该项研究设计合理、方法先进，结果有一定说服力，达到国内同类研究工作的先进水平。

（本刊讯）

Changes in Serum Copper, Zinc and Vitamin A, E and Their Significances in Patients with Acne Vulgaris
—Observation of Therapeutic Effect in 56 Cases Treated with Zhenye Baishecao Tang (增液白蛇草汤)

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The authors have treated 56 cases of acne vulgaris with the prescription Zhenye Baishecao Tang (增液白蛇草汤). It is to be prepared with several constituents, including Radix Ophispogonis 15～20 gm, Radix Rehmanniae 15～20 gm, Radix Scrophularia 10～20gm and herba Oldenladia 20～30gm. Their dosage should be either increased or decreased according to age, sign and symptoms of the patient. Some Chinese herbal drugs are to be added for controlling other signs. The prescription was effective to all 56 patients, 25 cases were completely cured, the rest were greatly improved.

In order to explore the pathogenesis of acne vulgaris in 38 cases serum copper and zinc levels were detected by atom absorption spectrophotometric method ; while the fatty-soluble vitamin A, E were detected by fluorescence spectrophotometric method. The results show that serum levels of zinc, vitamin A and E were significantly lowered statistically, as compared with those of the control ($P<0.001$).

The tongue figures and pulse conditions were observed and then analyzed in 37 cases. From the viewpoints of TCM-WM, the authors claim that the pathogenesis of acne vulgaris might be caused by the dysfunction of spleen and stomach. Moreover, the lowered sreum levels of zinc, vitamin A and E might serve as some aspects of their objective standard of its function. As we know that the function of the stomach is active in wetness but depressed in dryness evil, under such circumstances the dryness evil of stomach might characterize the normal function of spleen. The excessive dryness evil of spleen then exerts influence on the lung, keeping in dryness from heat. If the excessive dryness evil in lung exists for longer time, it might lead to "fire" evil. As skin and hairs were controlled by lung, so acne eruptions appear on the face. Hence spleen and stomach form an exterior and interior relation, and so do the lung and large intestine ; therefore in clinical practice the above four organs are always responsible for acne. On the other hand, the use of moisturized herbal drugs in the treatment of acne vulgaris often gives satisfactory effects, thus confirming TCM theories.

(Original article on page 169)

Immunopharmacological Effect of Acinidia Chinensis Polysacchacide

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This paper reports the immunomodulatory action of the polysaccharide extracted from the root of Actinidia chinensis (ACPS). Mice were given ACPS daily intraperitoneally for 1～2 weeks, and their immune functions were examined. The results were as follows: (1) ACPS could significantly enhance the natural killer (NK)-cells at 10 and 13 days after administration. (2) It might enhance the phagocytic function of macrophages. (3) It could markedly increase the number of SRFC (specific rosette forming cell), but there was no change on antibody forming cells at all. (4) It might actively restore the delayed-type hypersensitivity reaction from the suppression by cyclophosphamide. The above-mentioned results indicated that ACPS acts as an effective immunomodulating agent. It may probably be of great benefit for hosts to strengthen their anti-viral and anti-neoplastic activities.

(Original article on page 171)

Chronomorphological Effects of Needling "Yong Quan Point" on Some Rats' Organs

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78 maturated male rats were divided into three groups randomly: The first group (24 rats) was untreated control group which were sacrificed at 7:00, 13:00, 19:00 and 1:00; second group (24 rats) was acupunctured on "Yong Quan Point" for 25 minutes, then killed at the same time as control; while the third group (30 rats) was injected 20 μg/kg ACTH 1-17 subcutaneously at 7:00, then slaughtered also at the same time as the control, but one additional kill was performed 24 hours after the injection. After sacrificing, the internal organs were removed and put in suitable fixatives. The following substances and processes were observed: (a) The amount of vitamin C in adrenal cortex, (b) the Golgi apparatus in epithelium of distal renal tubule, (c) the amount of hepatic glycogen, (d) the proportion of binucleated cell to mononucleated one, and (e) the spermatogenesis. Results: (1) In control group, from (a) to (e), all of the items had the circadian rhythm, with the peak at 19:00, 13:00, 1:00 (mature sperms) respectively. The amount of hepatic glycogen of light period was lower than that of dark period . (2) In acupuncture group, the circadian rhythm of (a), (c), (d) were abolished by acupuncture, while the (b) and (e) remained. (3) In ACTH group, the circadian rhythm of (a), (b), (c) were abolished by the injection of ACTH, but that of (c) and (d) existed, the light period was lower than the dark period in former case, while in latter case, 24 hours after injection , the peak was reached. (4) The results of this experiment and their significance were discussed. (5) These results could be used in studying the "Zi Wu Liu Zhu" (子午流注), selecting acupoints based on matching them with two-hour periods traditionally divided) theory in traditional Chinese medicine.

(Original article on pape 174)